

五五

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Hypothetical model of the HCV replication cycle

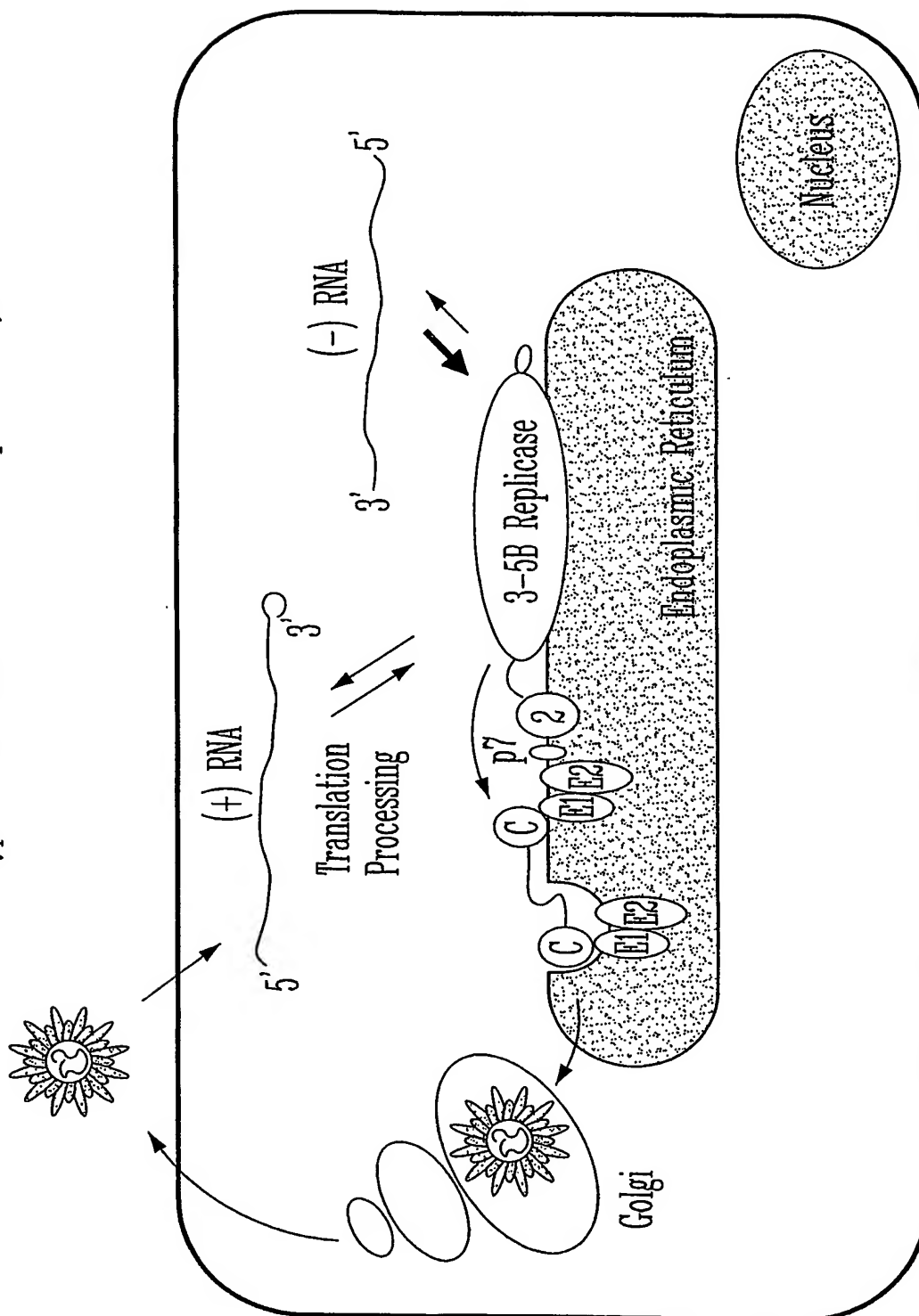


FIG. 2

Experimental Protocol.

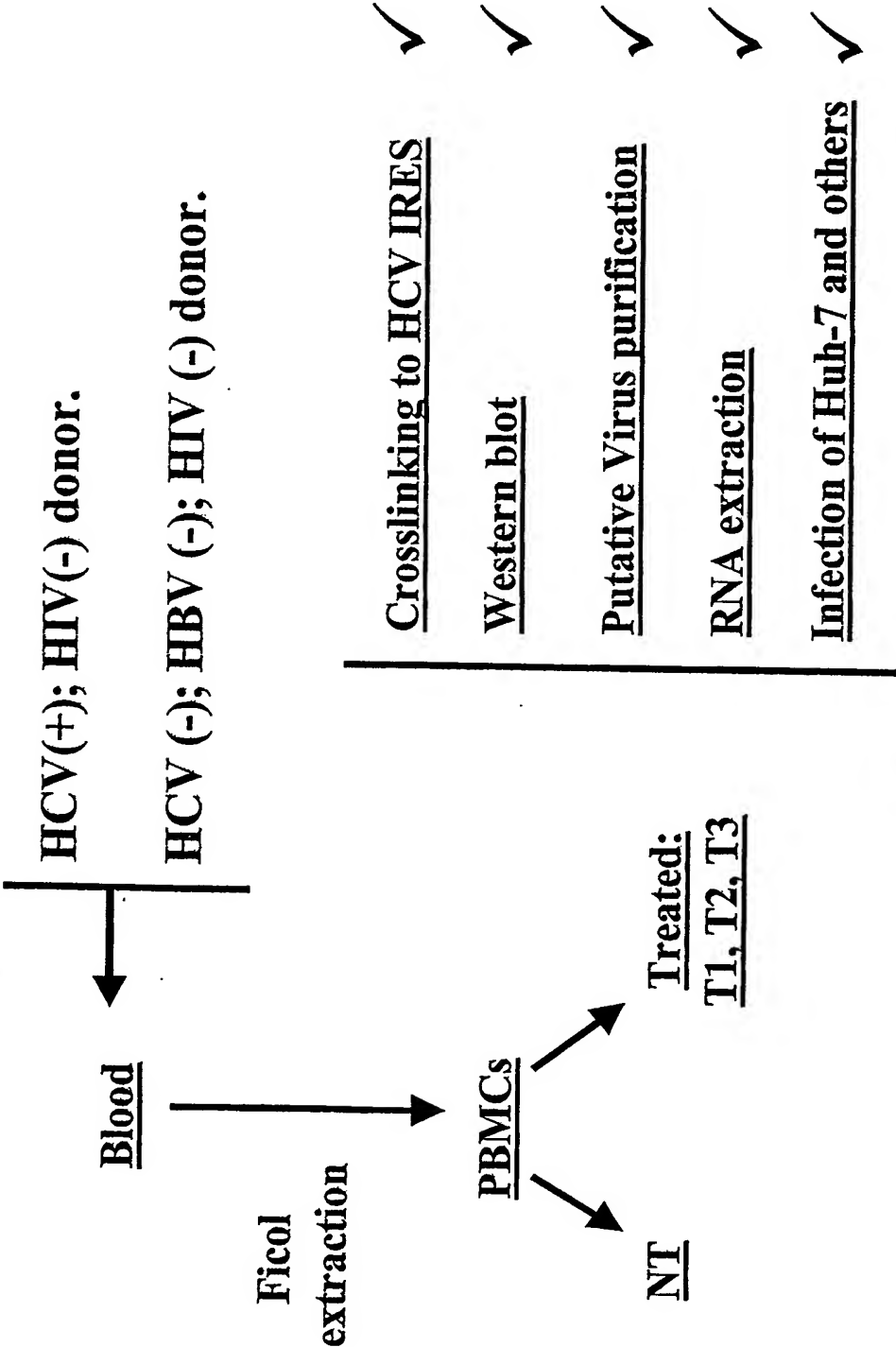
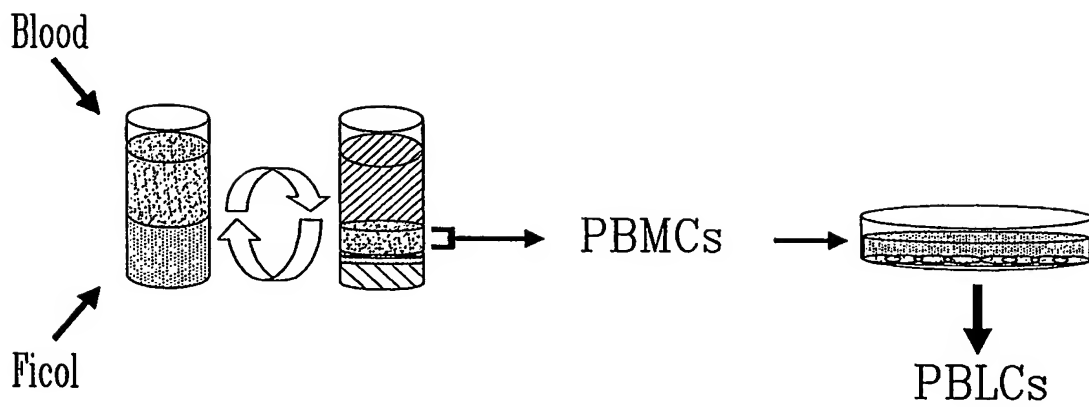


FIG. 3

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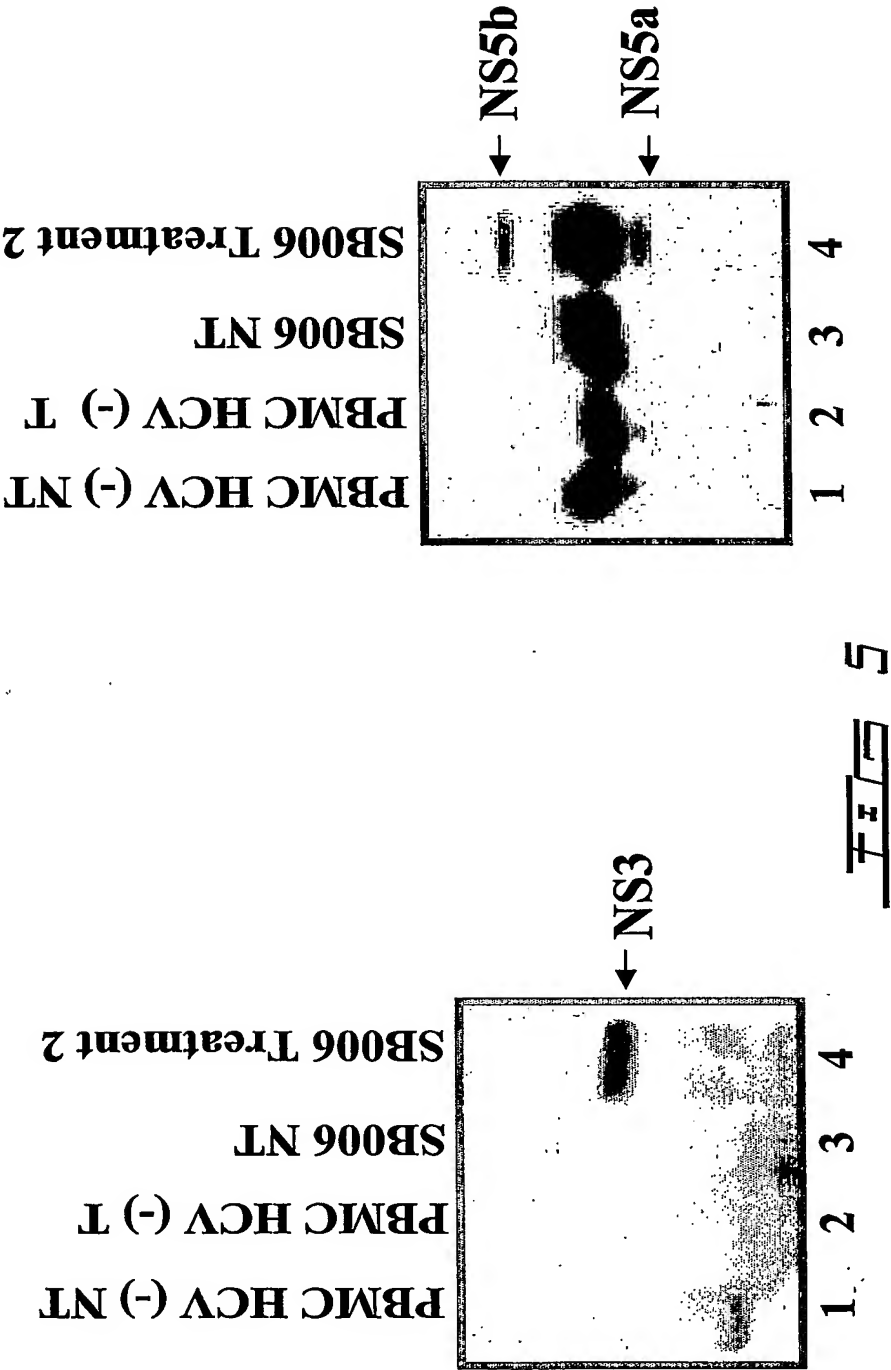
PBMC and PBLC purification from blood samples.

FIG. 4

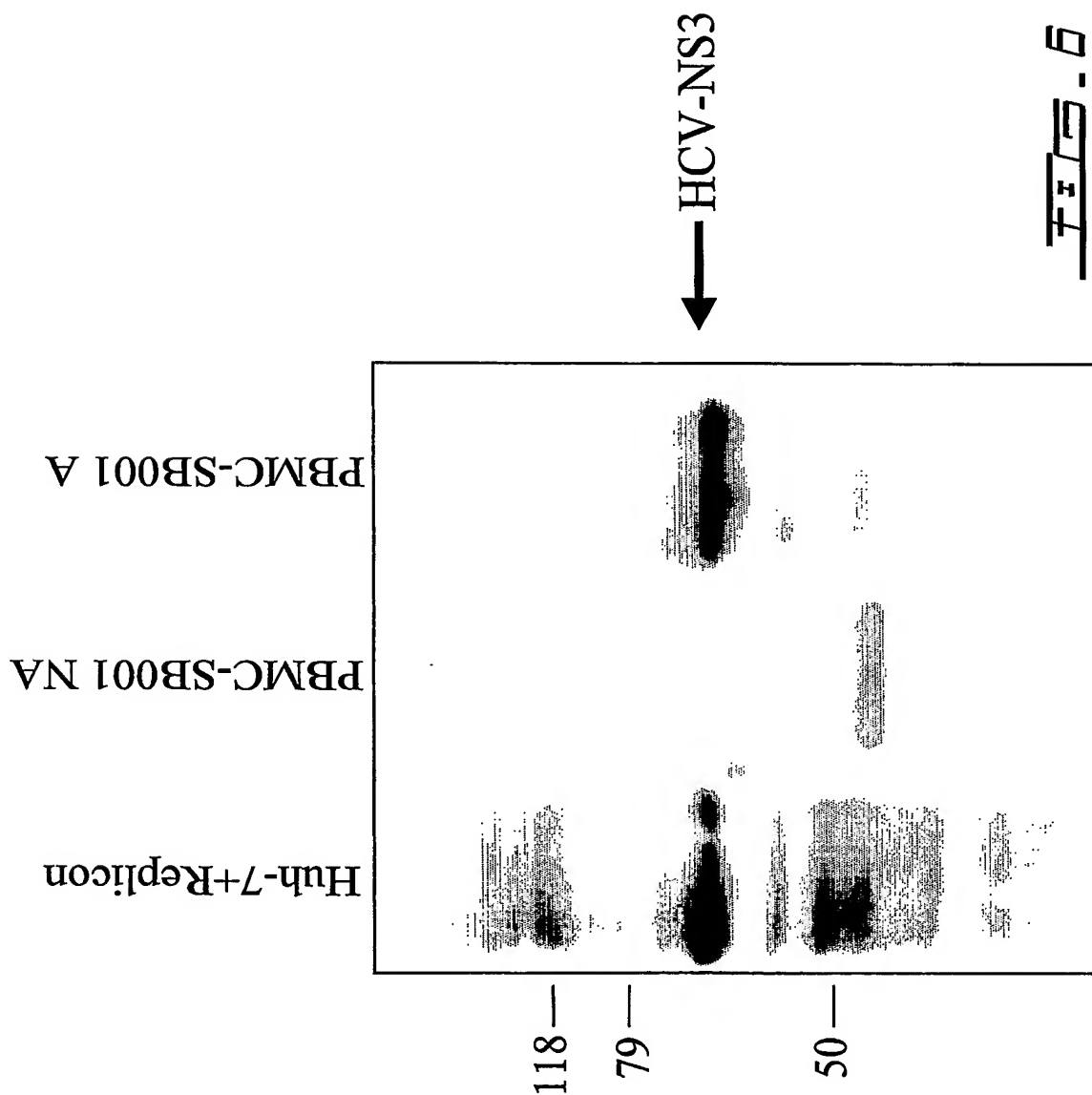
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Detection of HCV NS3 and NS5 proteins in cell extracts from Treated

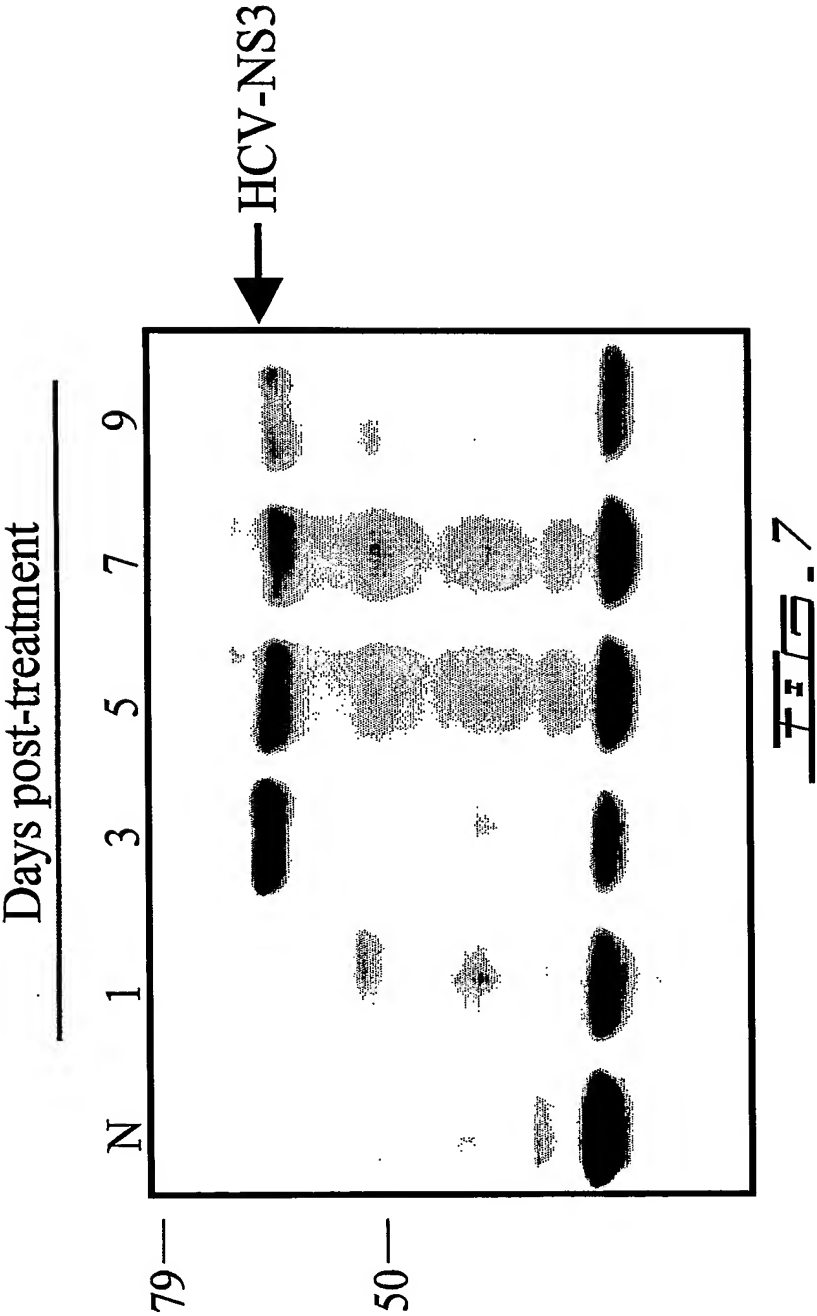
PBMC from an HCV (+) patient.
[Boeringeranti-NS3 polyclonal antibody]



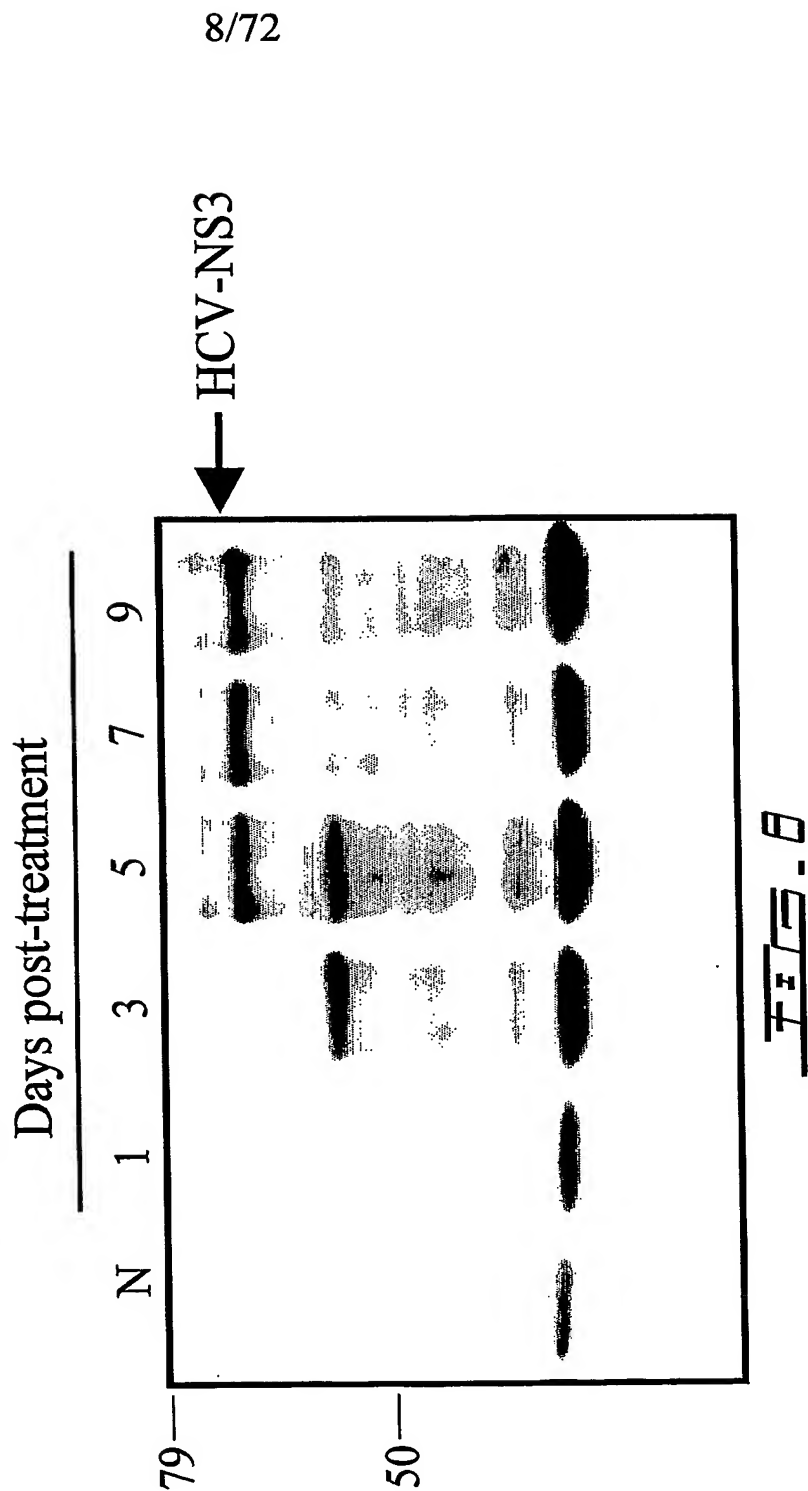
6/72



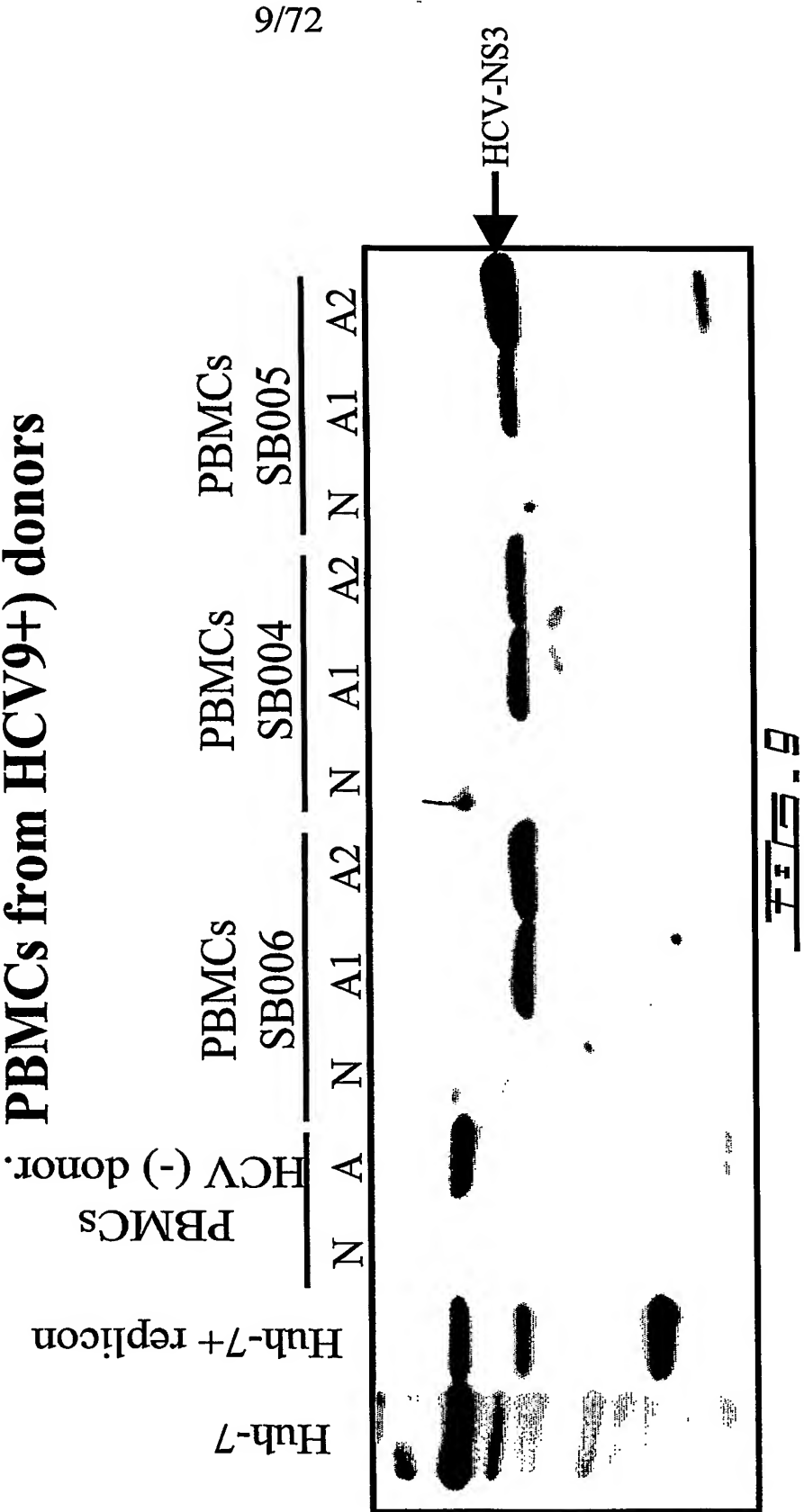
**Time course of HCV-NS3 detection:
PBMCs From patient MLL-001**



Time course HCV-NS3 detection: PBMCs from patient MLL-002



Detection of HCV-NS3 protein in treated (N3)
PBMCs from HCV9+) donors



Detection of virus like particles by scanning electron microscopy

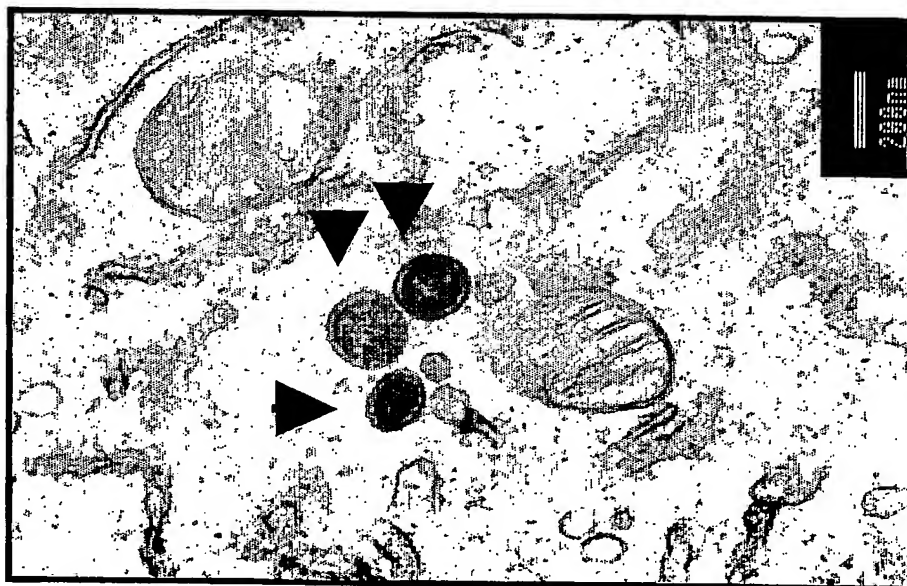


(-) Control

FIG. 10

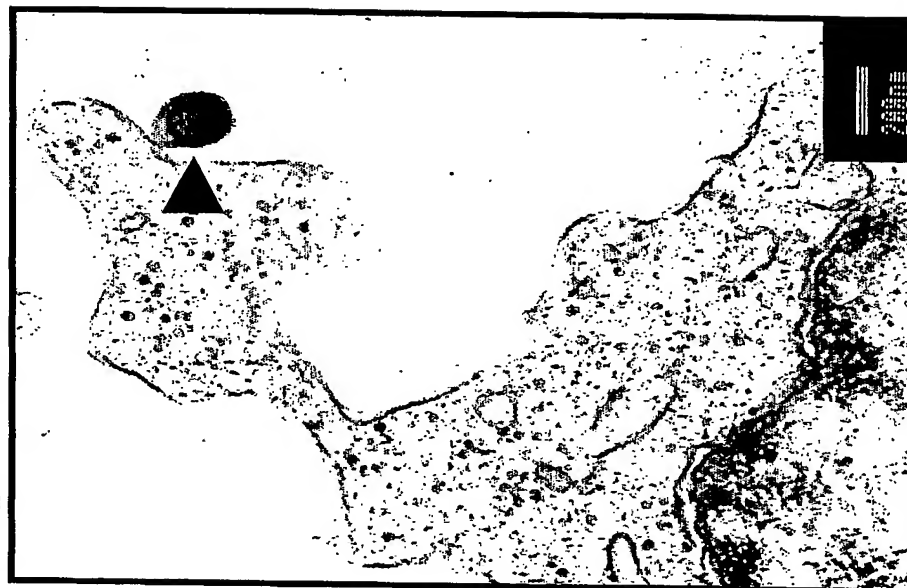
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**Electron microscopy of Activated PBLCs;
Detection of virus like particles**



200 nm

Figure 11



200 nm

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Virus partial purification.

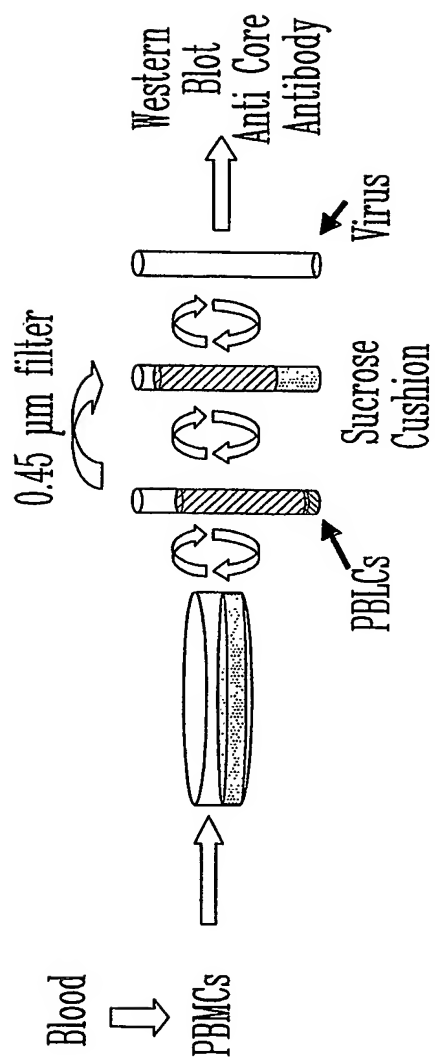
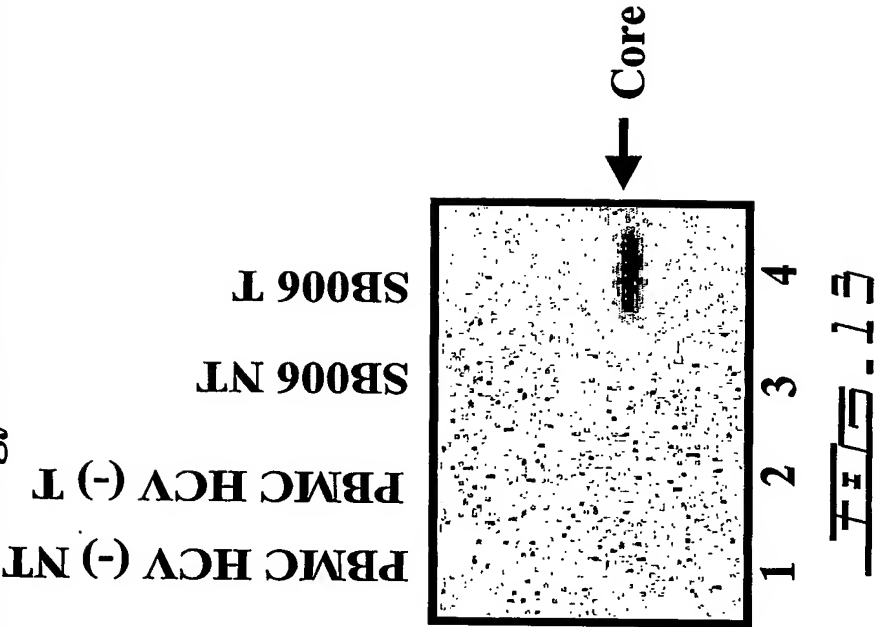


FIG. 12

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Detection of HCV Core protein in supernatant of treated
PBMC from an HCV (+) patient.
[Maine biotechnology anti-Core monoclonal antibody]

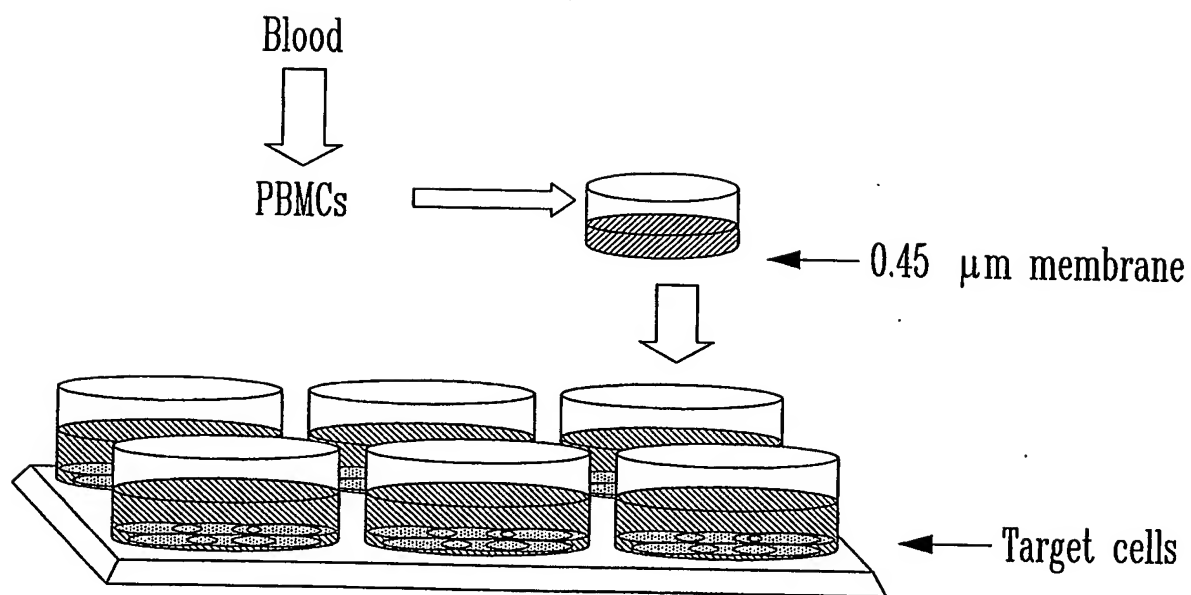


RNA Quantification I (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant
<u>After 4 days</u>		
SB004 NT	2x10 ³	No
SB004 T	2x10 ³	Yes
SB006 NT	1.8 x10 ³	No
SB006 T	2x10 ²	Yes
<u>After 20 days</u>		
SB004	0.00	
SB006	0.00	<u>Fig. 14</u>

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Infection assay; co-culture

FIG. 15

Infection of MT-4 cells
RNA Quantification II (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant	HCV RNA In MT-4
<u>After 10 days</u>			
SB001 NT	13	No	0.00
SB001 T	12	Yes	1600
<u>After 20 days</u>			
SB001	0.00		0.00
SB001	0.00		0.00

FIG. 16

Co-culture of Huh-7 and HCV (-) PBMCs.

- 1- Huh-7
- 2- Huh-7 + PBMCs HCV (-) NT
- 3- Huh-7 + Treatment
- 4- Huh-7 + PBMCs HCV (-) T

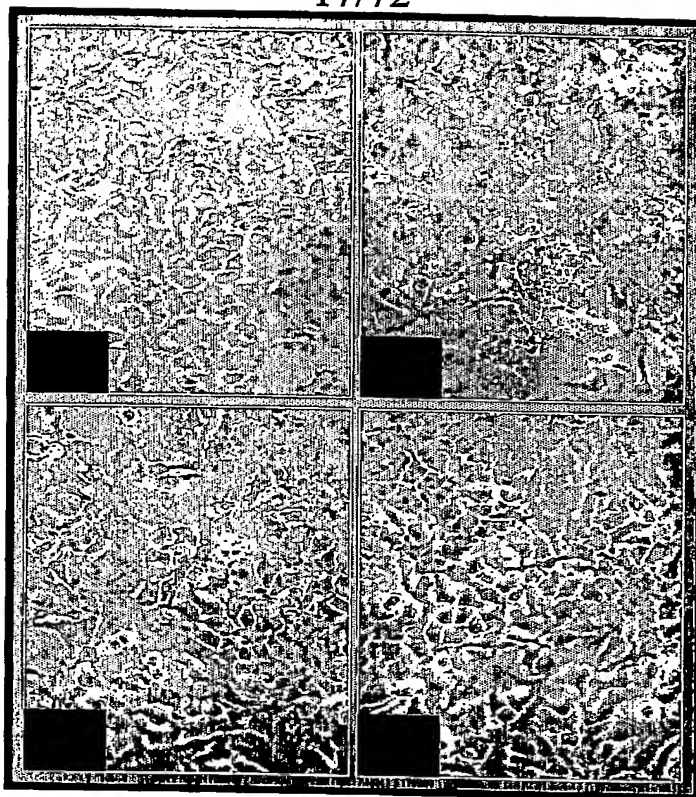
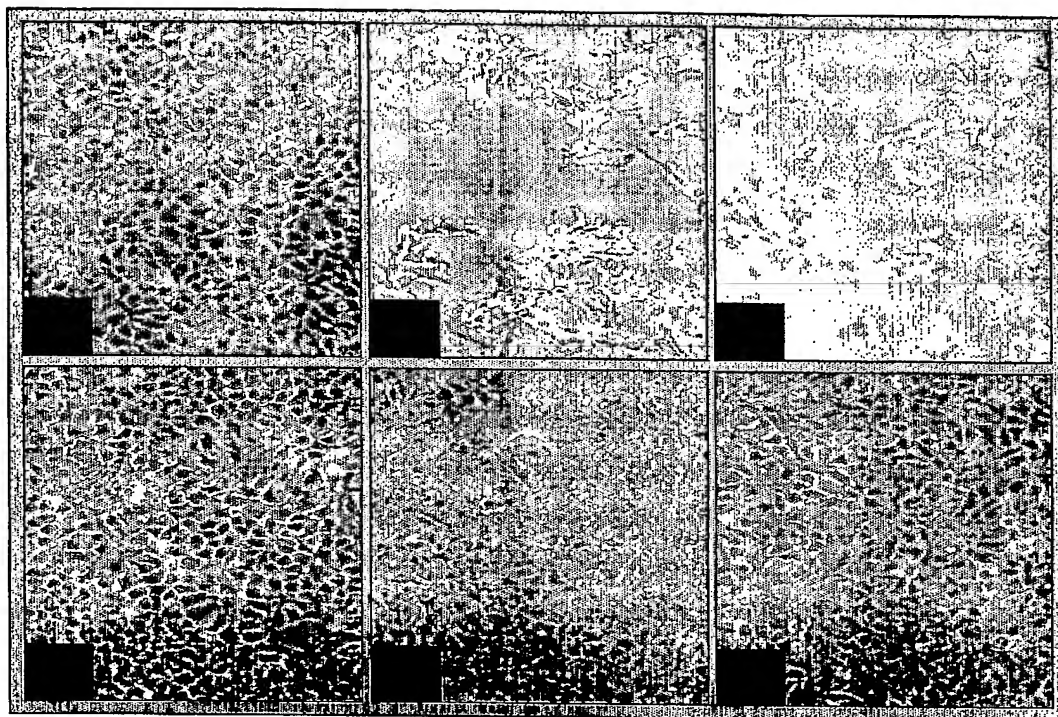


FIG. 17

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Co-culture of Huh-7 and HCV (+) PBMS° Cs (SB006).

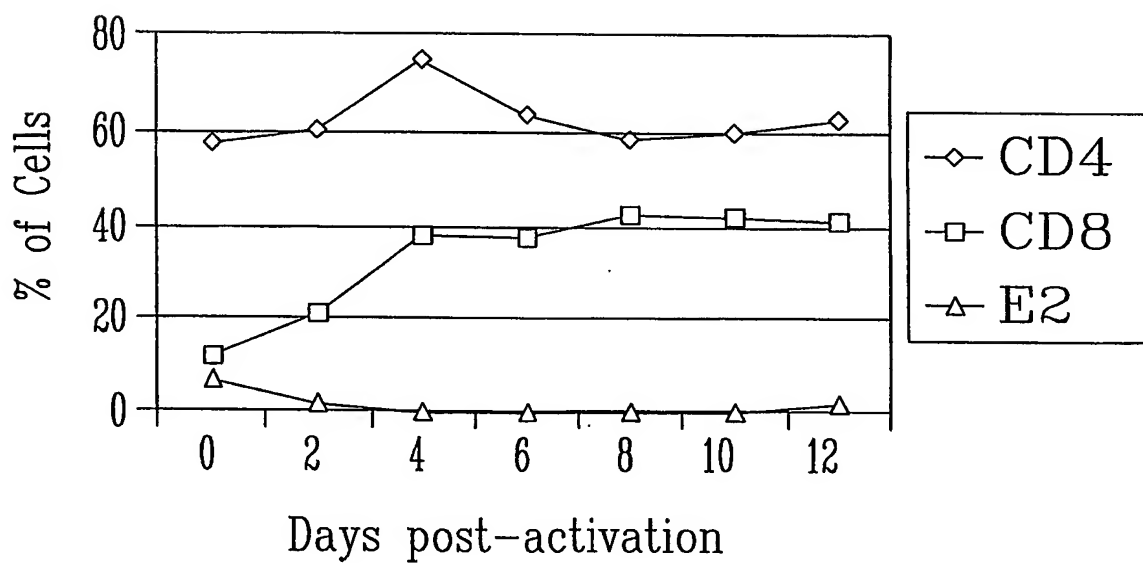


1. Huh-7
- 2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
- 5-6. Huh-7 + PBMCs HCV (+) T

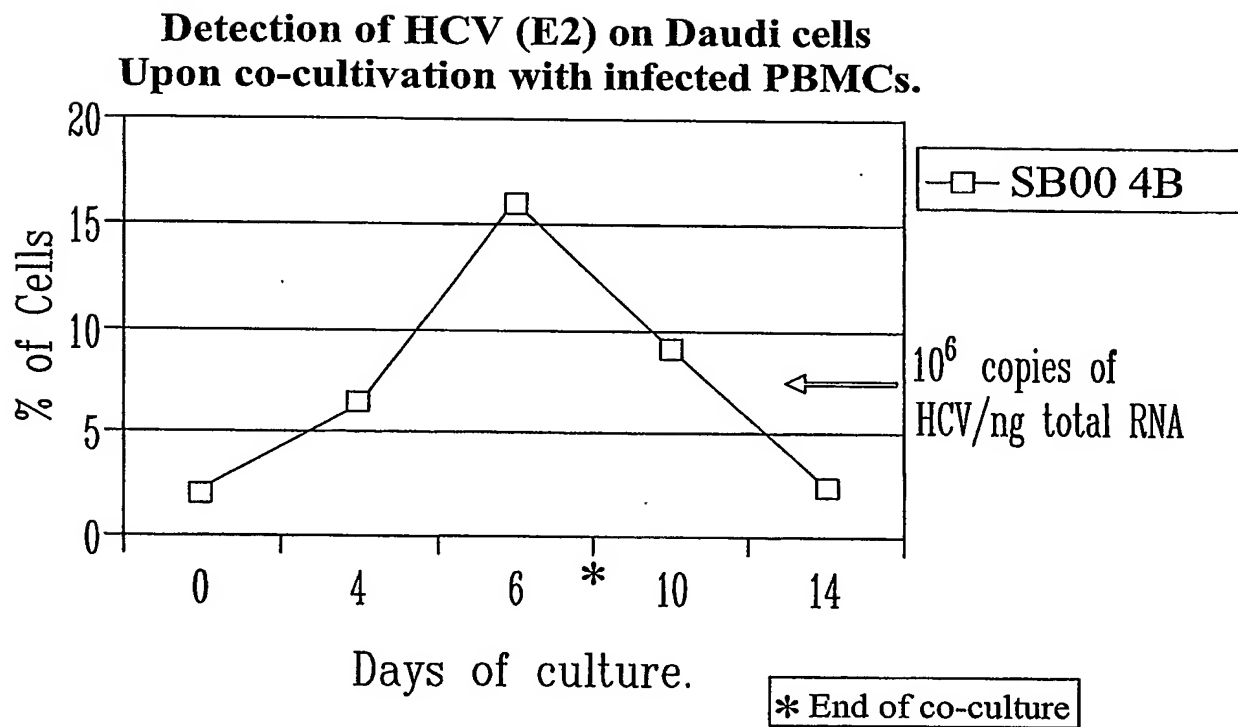
Fig. 18

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PHA Activation of PBMCs from patient SB004;
HCV is not in T cells

FIG - 19

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Fig. 20

Comparison of different activation treatments;
PBMCs from donor MLL-010

		T+B cells				Treatment			
		T cells (T1)				B cells (T2)			
		(T3)				(T3)			
N	2	4	8	12	2	4	8	12	4
		Days				Days			

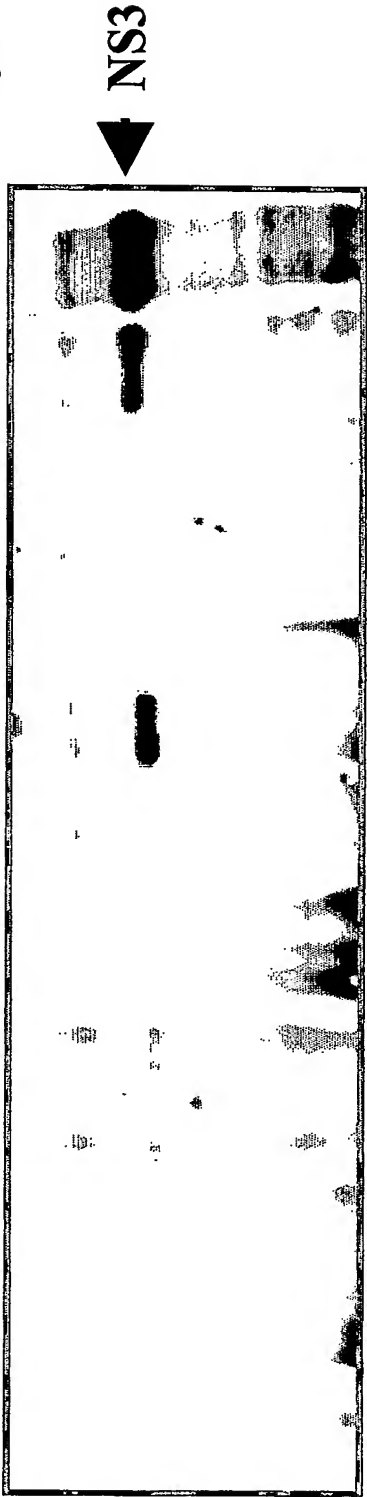


FIG. 21

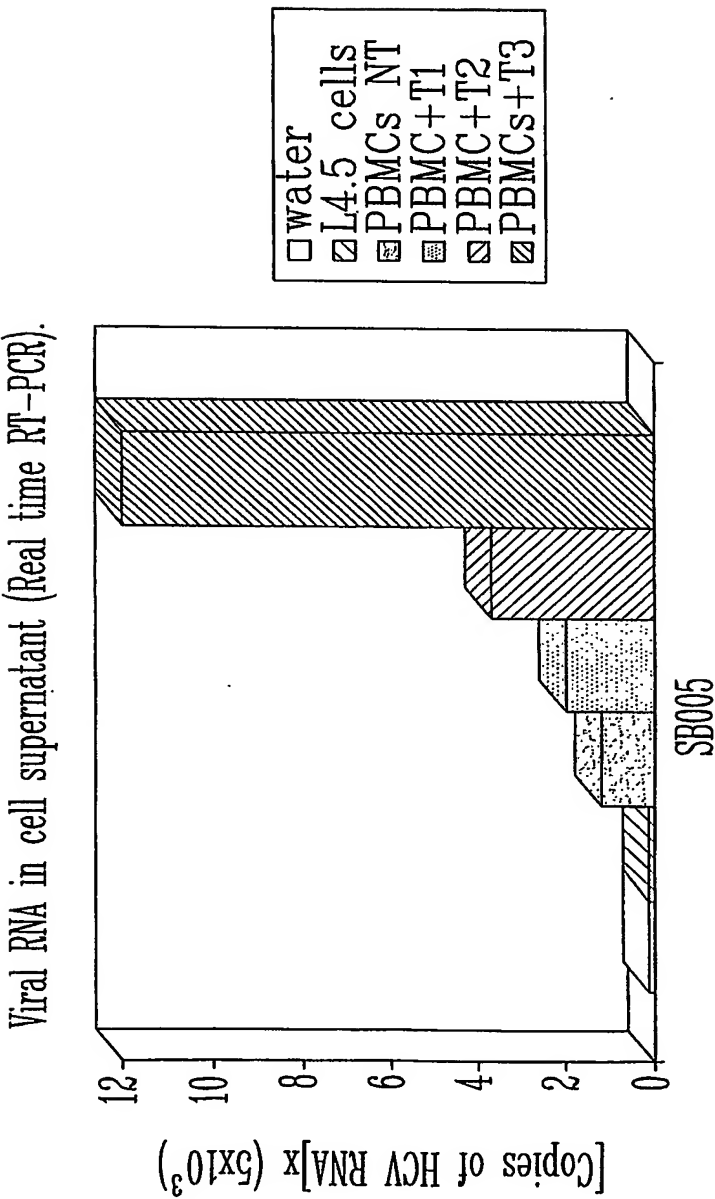


Fig. 22

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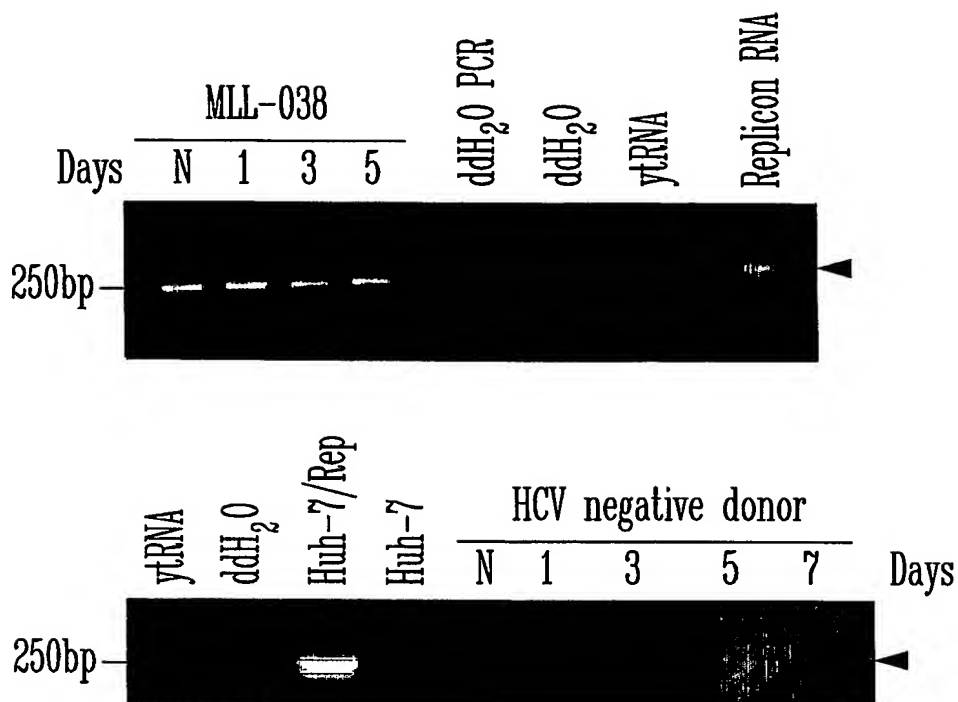


FIG. 23 A

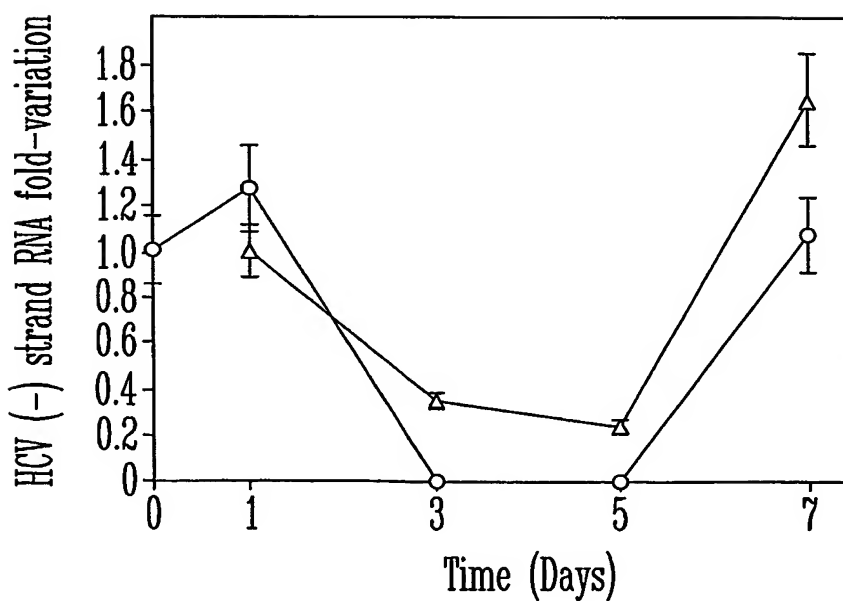


FIG. 23 B

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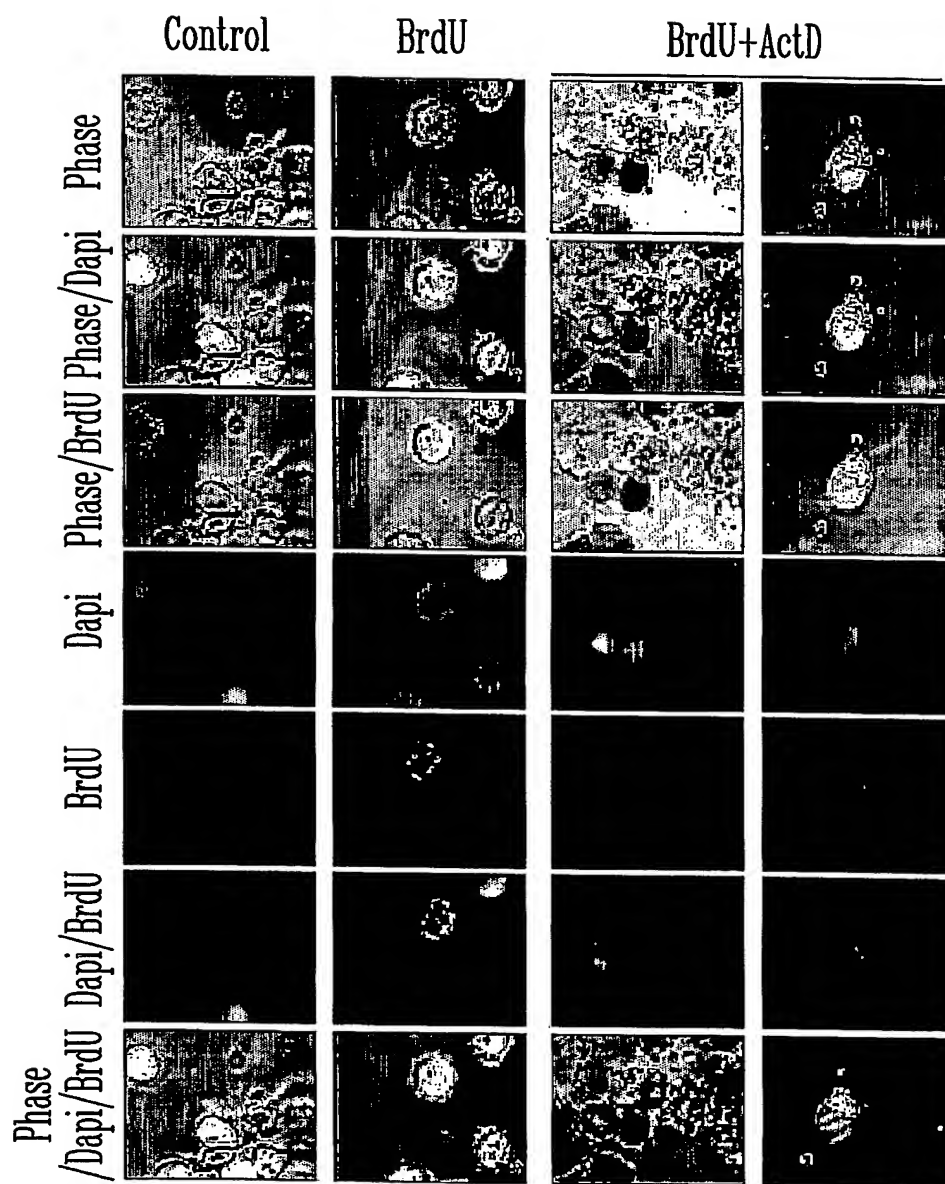


FIG. 23C

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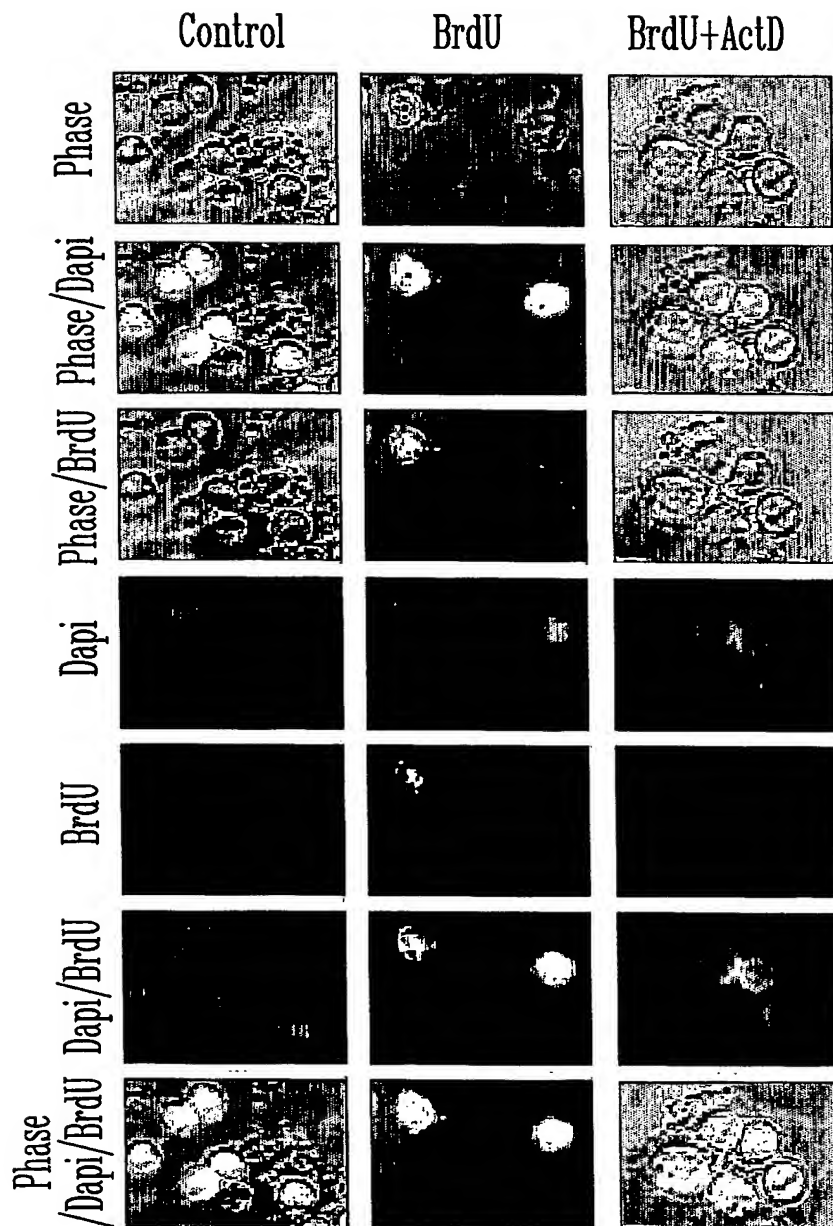
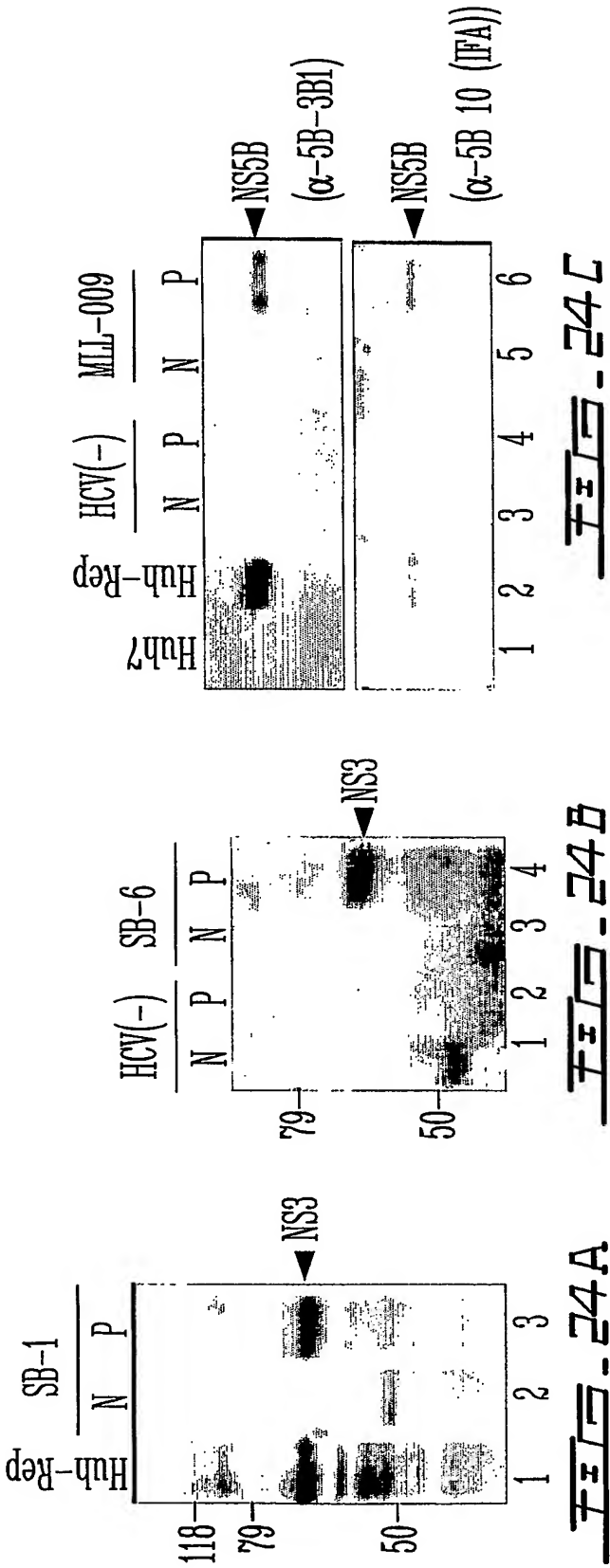


Fig. 23D



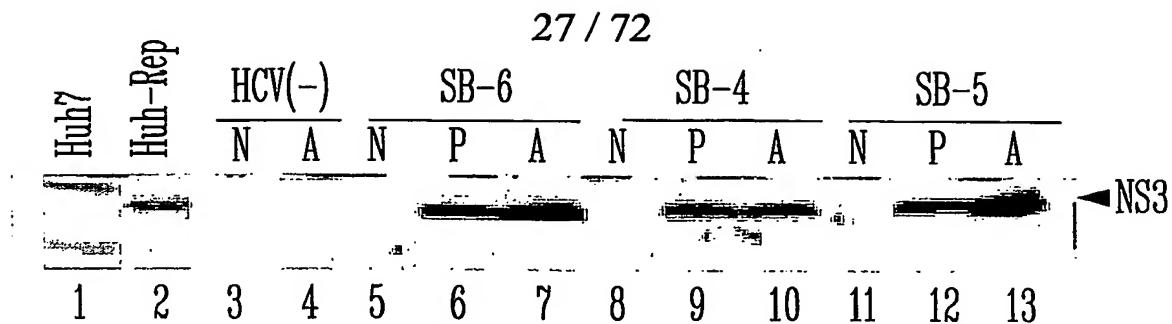


FIG. 24 D

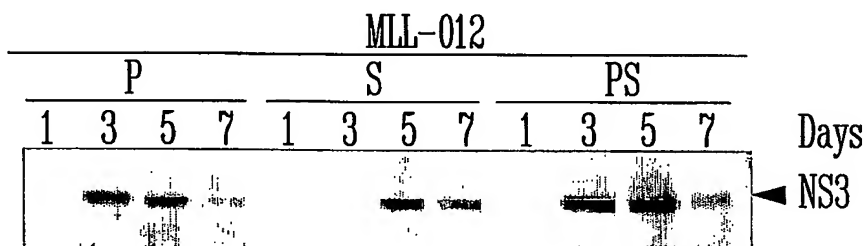


FIG. 24 E

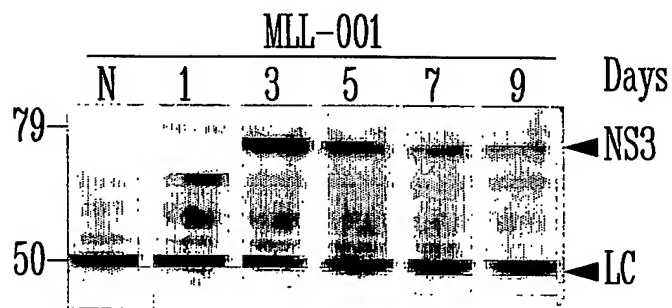


FIG. 24 F

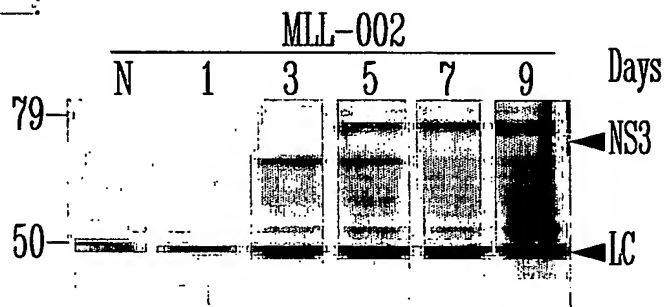


FIG. 24 G

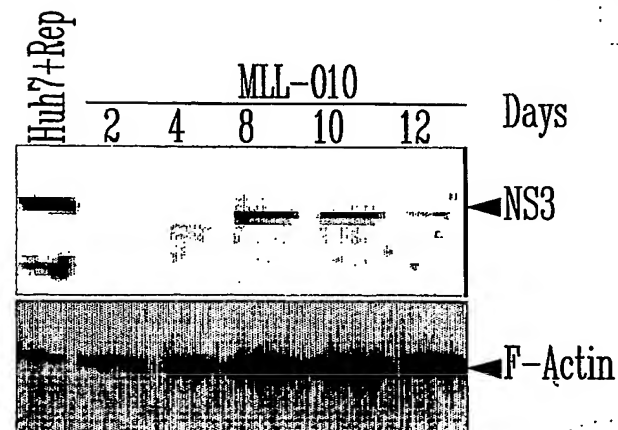


FIG. 24 H

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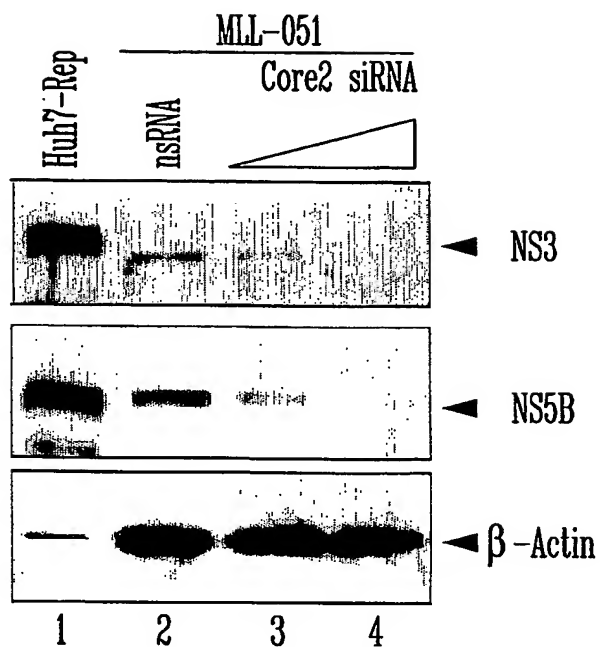


FIG. 24I

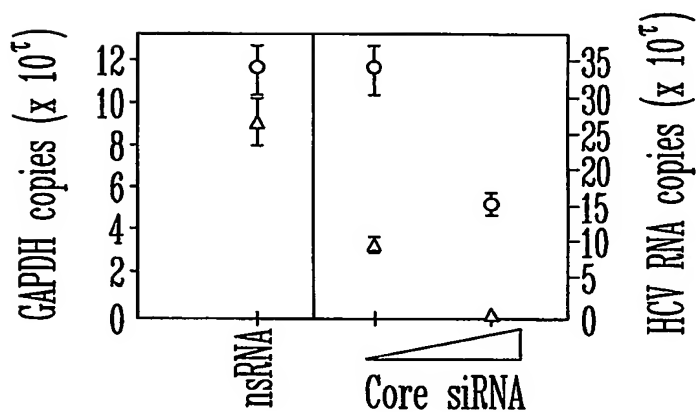


FIG. 24J

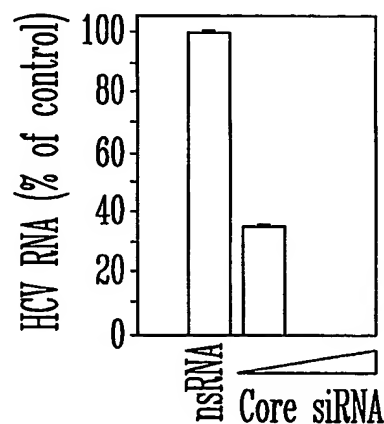


FIG. 24K

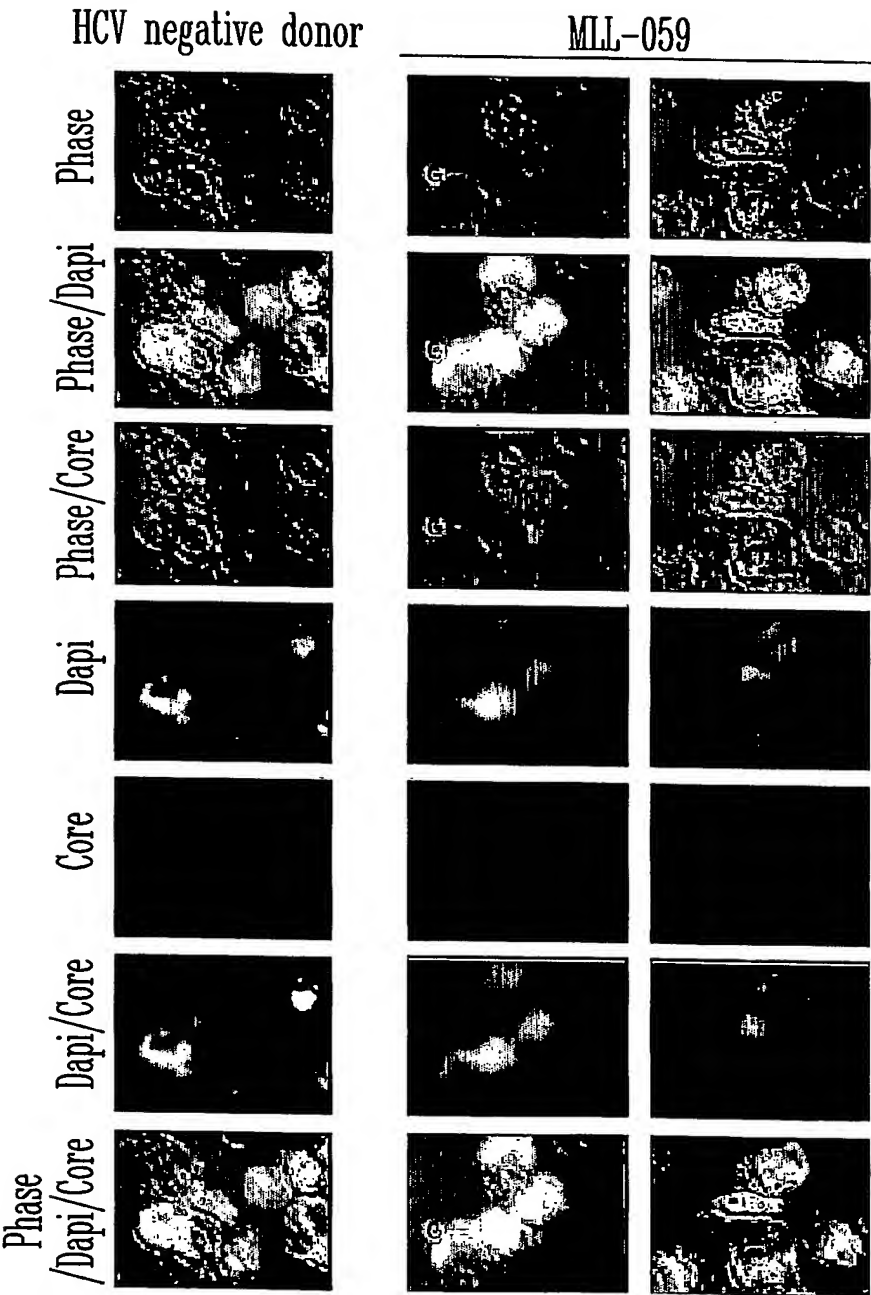


Fig. 25

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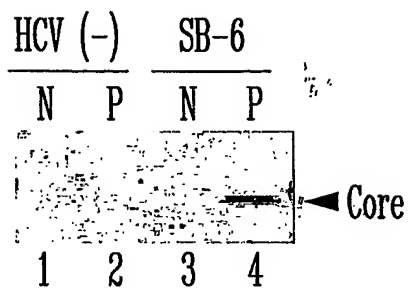


FIG. 26A

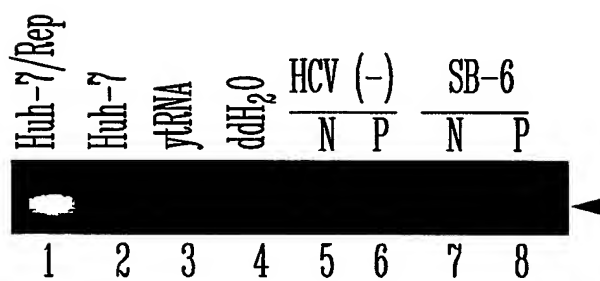
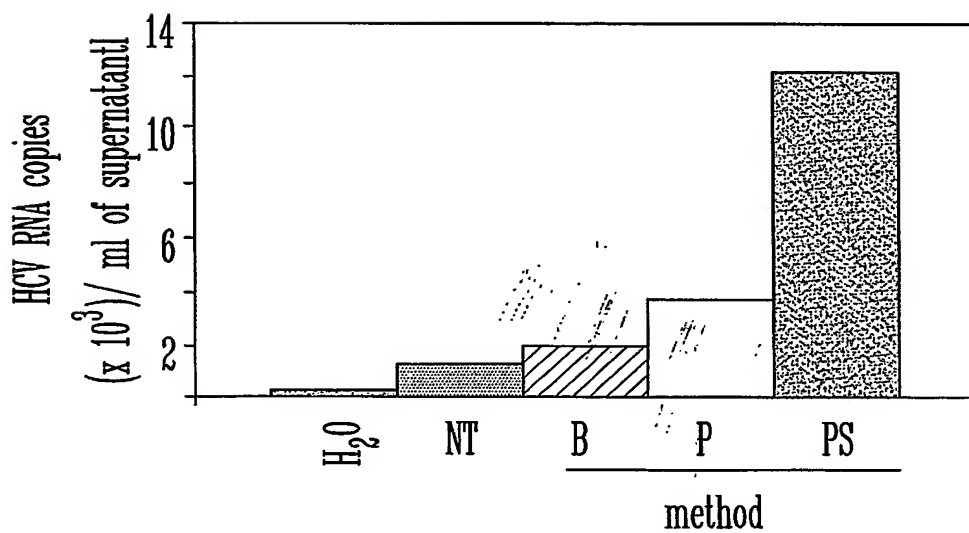


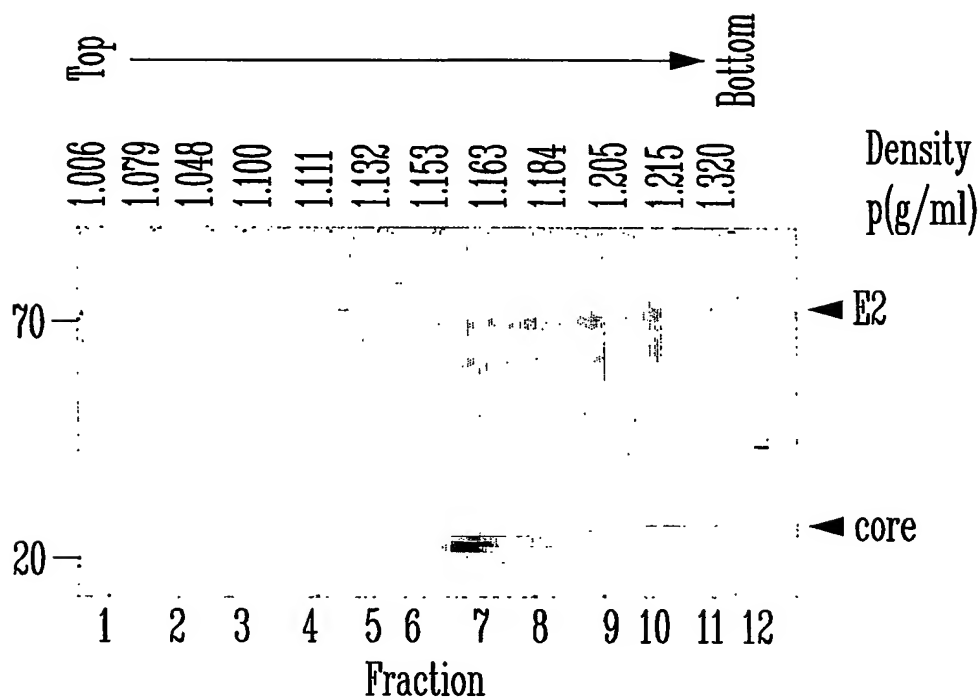
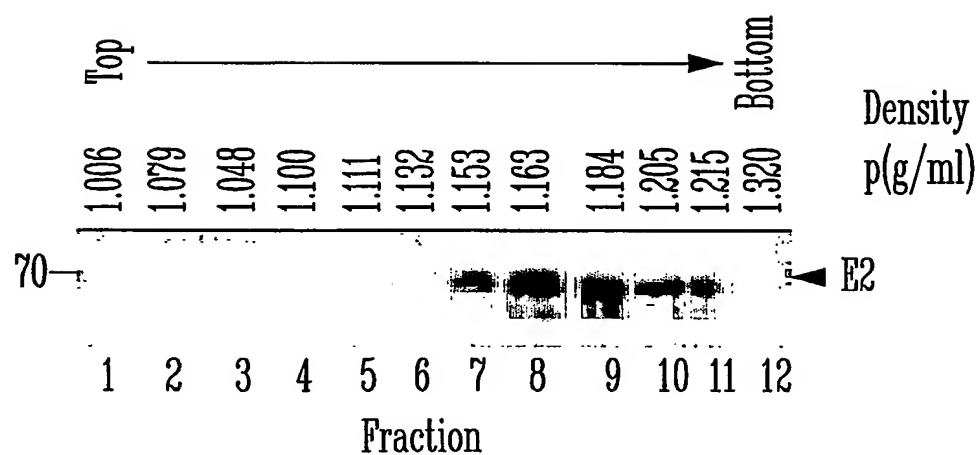
FIG. 26B



SB-5

FIG. 26C

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FIG. 26DFIG. 26E

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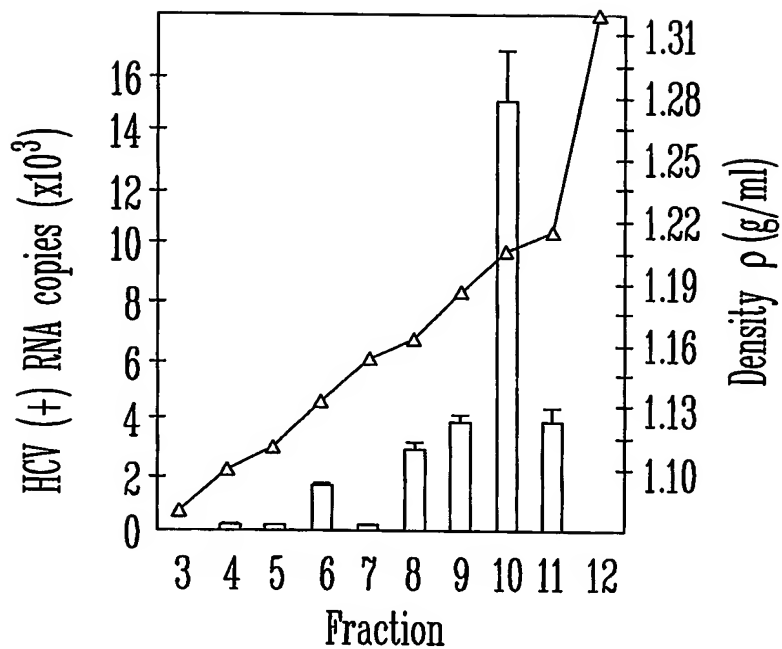


FIG. 26F

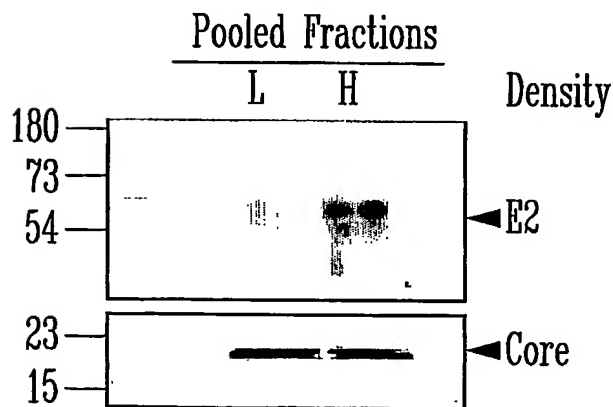


FIG. 26G

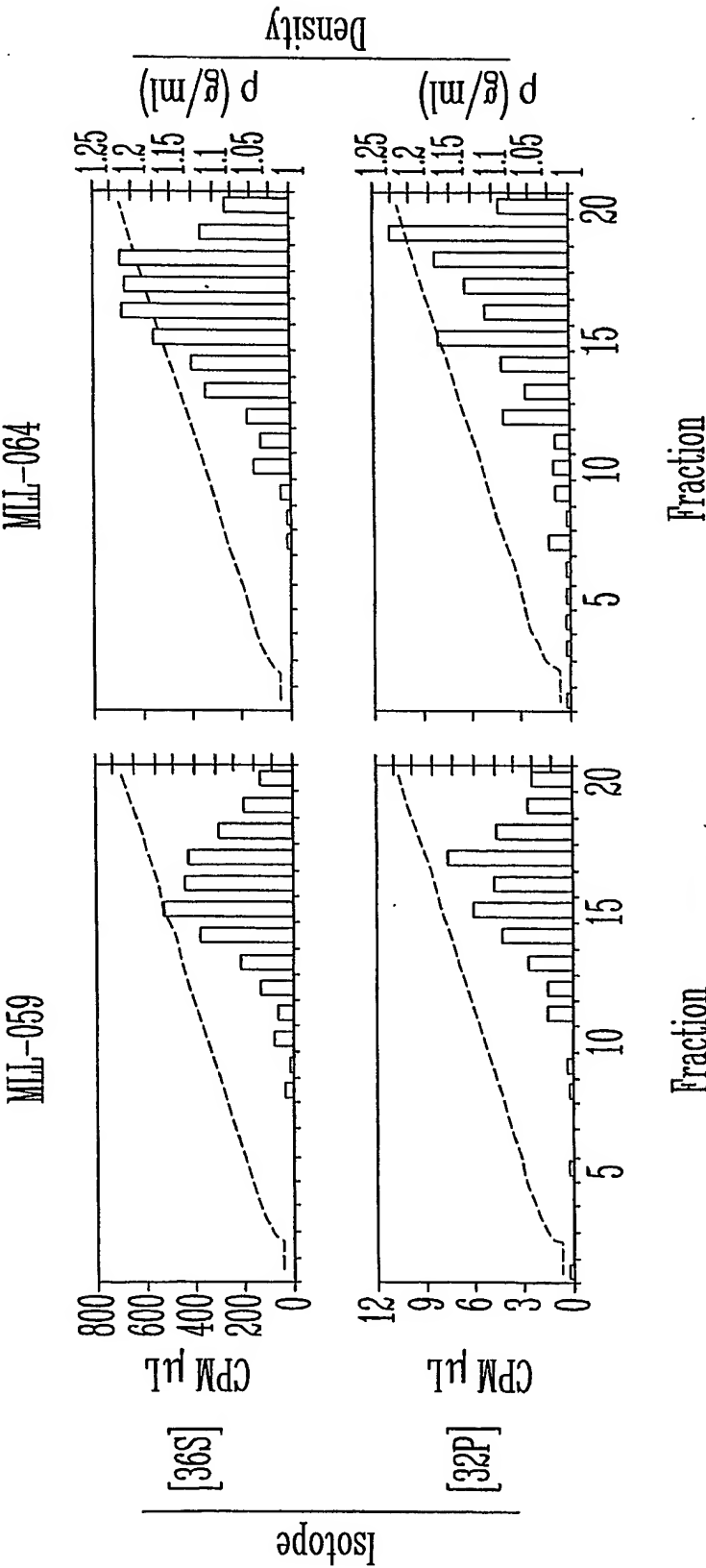


FIG. 25H

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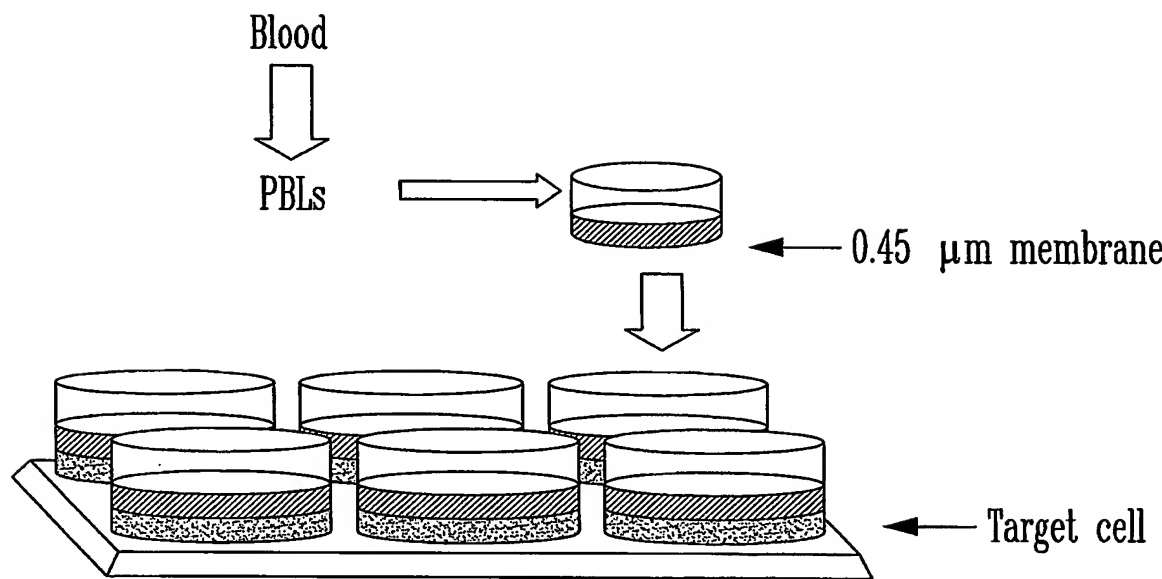


FIG. 27A

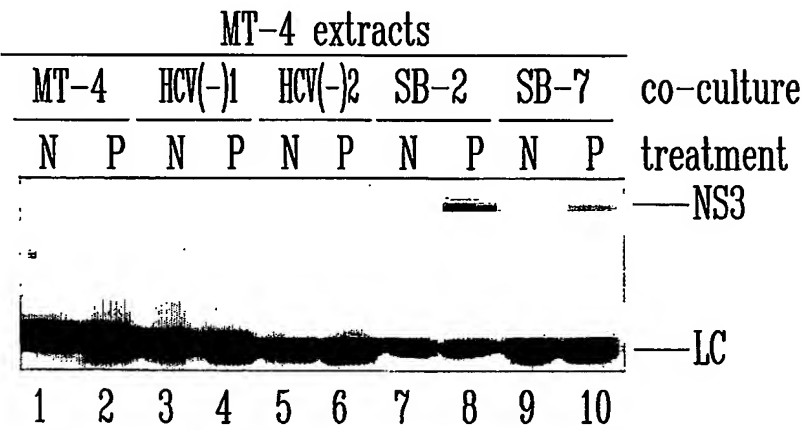


FIG. 27B

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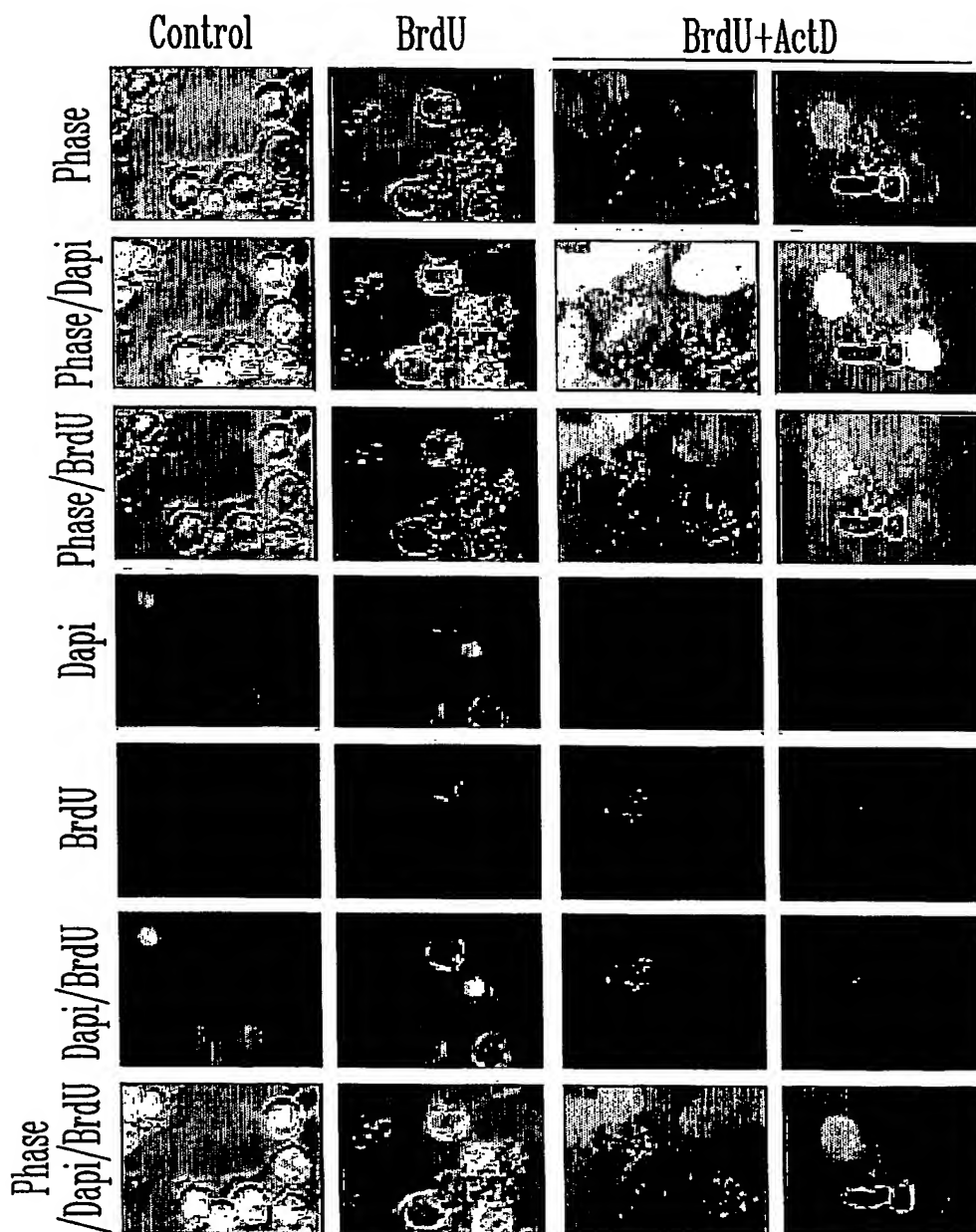


FIG. 28

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HCV Replication Cycle

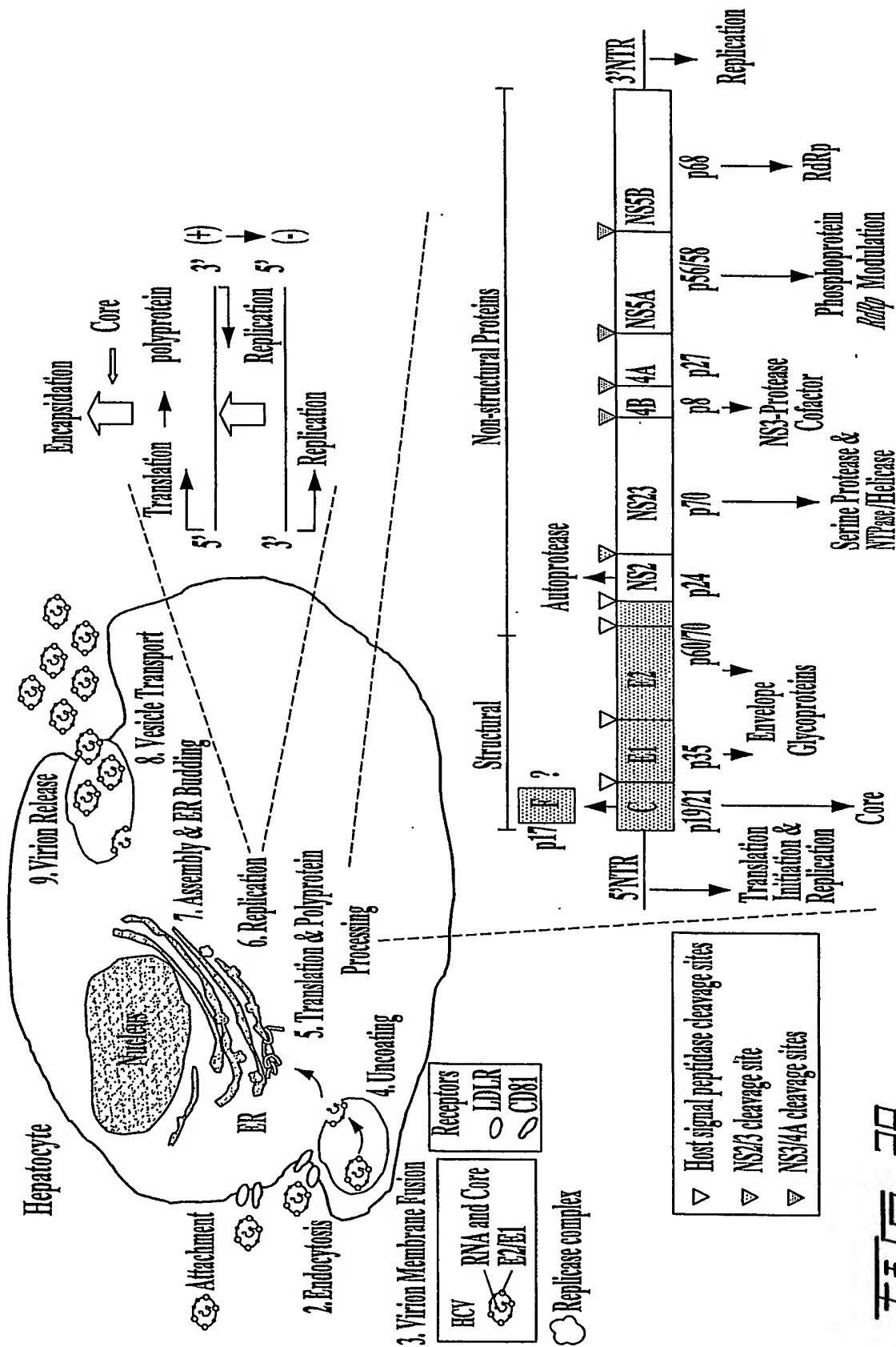


FIG. 29

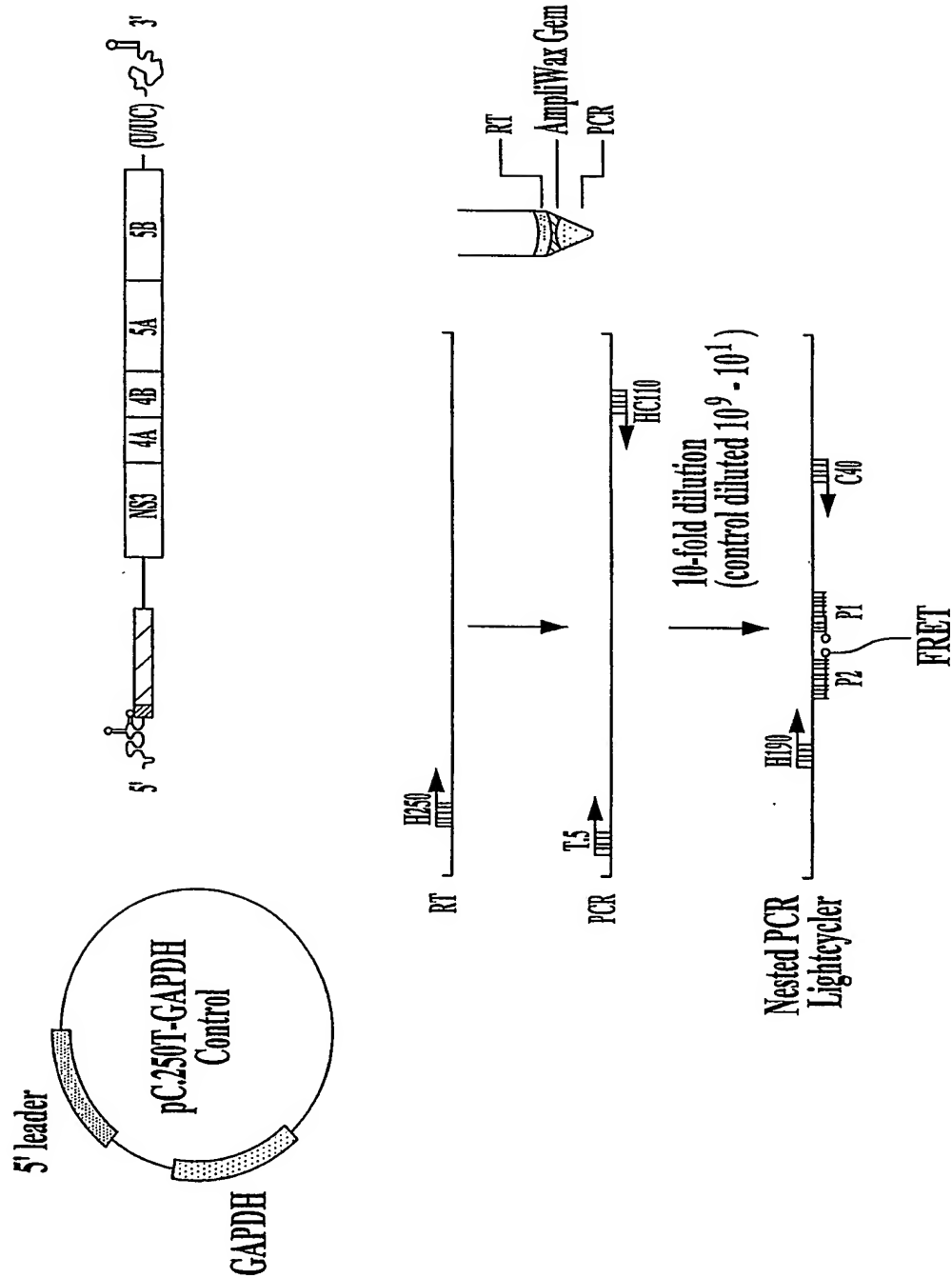
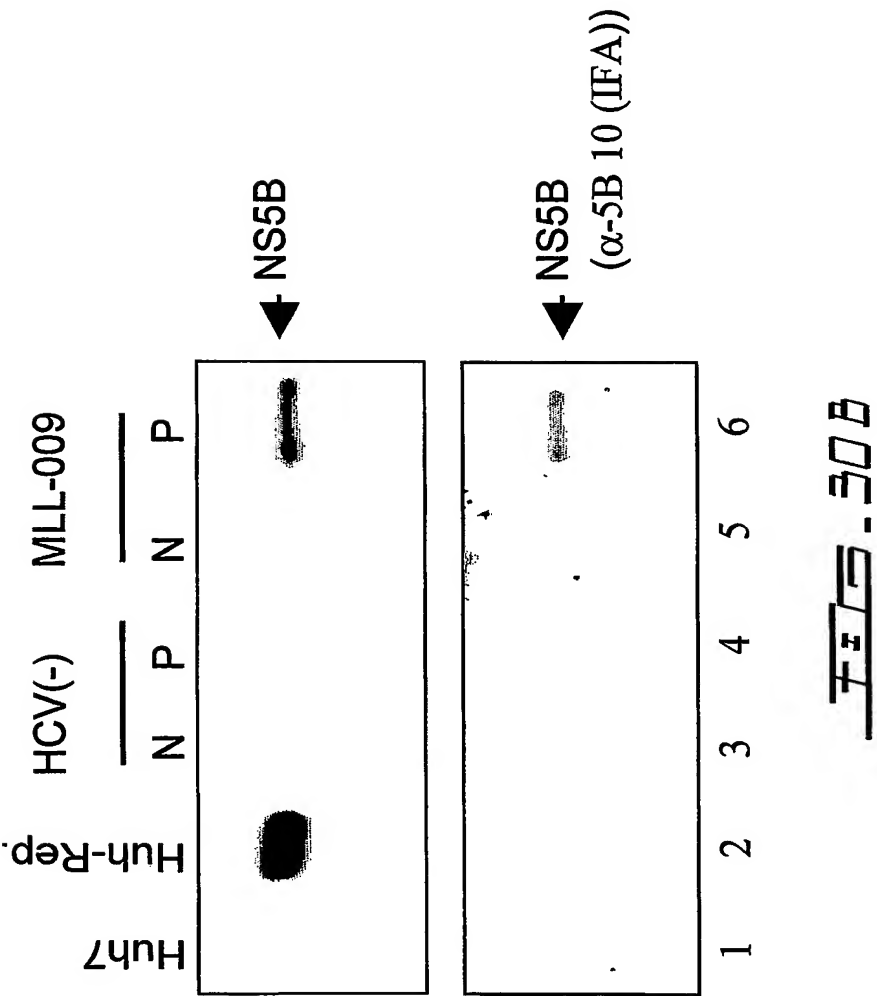


FIG. 30A

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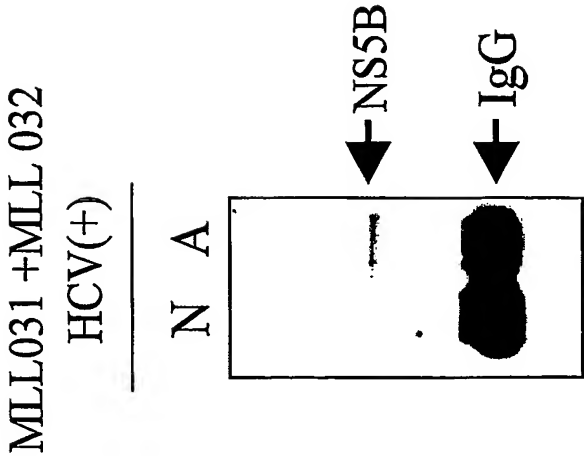
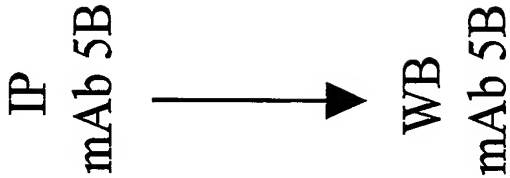
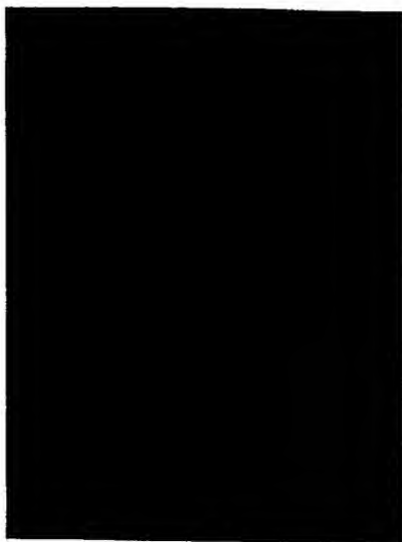


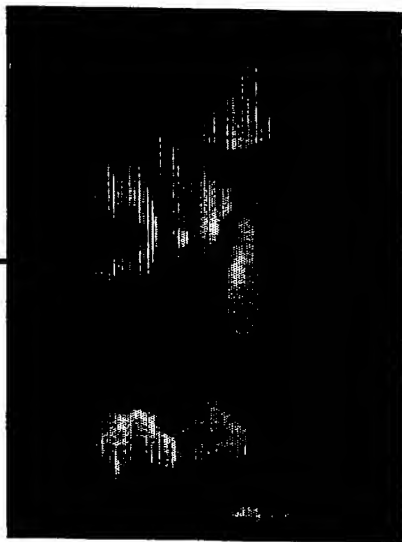
FIG. 31

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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



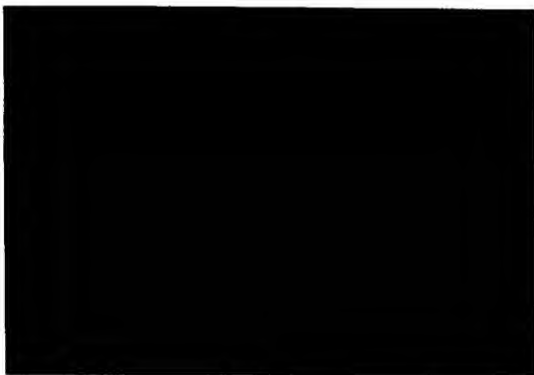
FIG. 32

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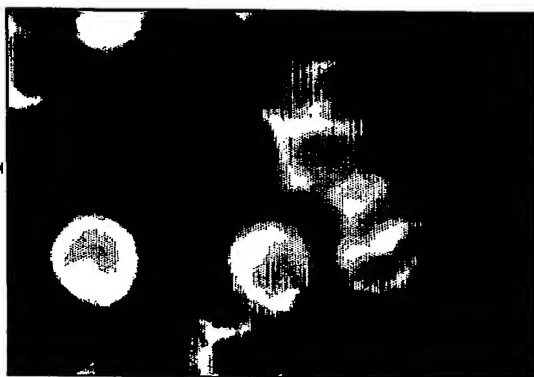
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FIG. 33

Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core

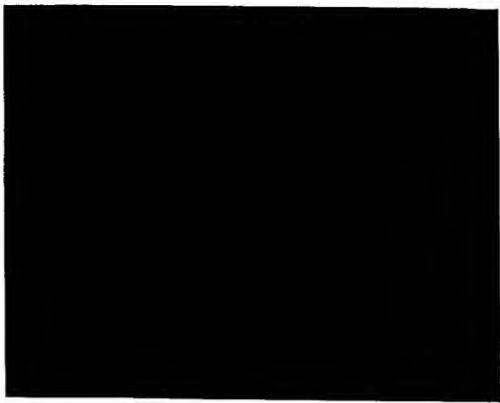


Dapi/Anti-Core

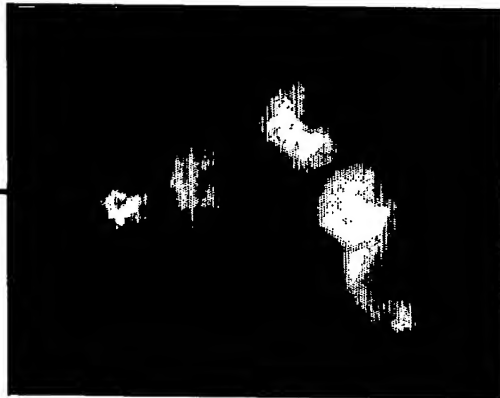


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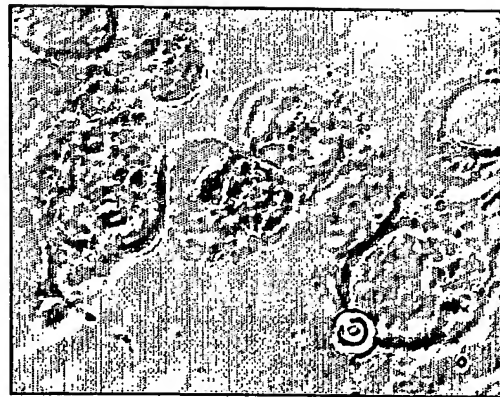
Anti-Core



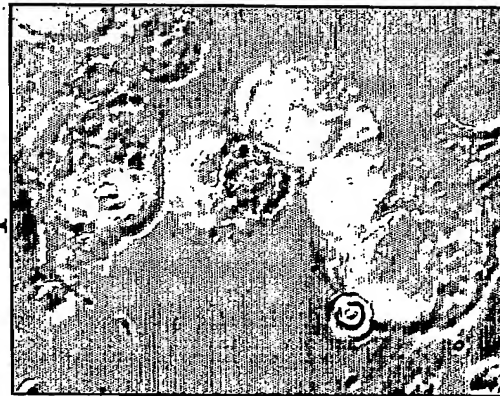
Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

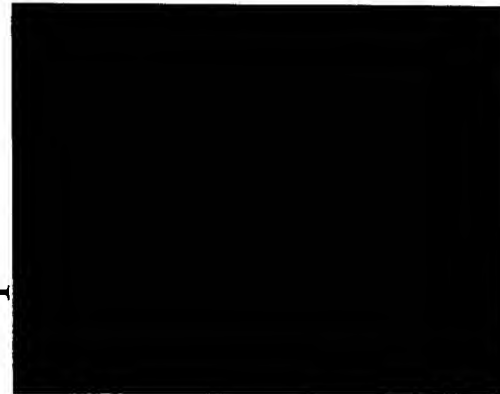
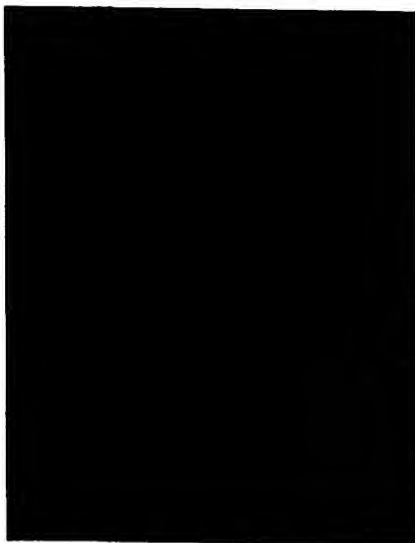


FIG. 34

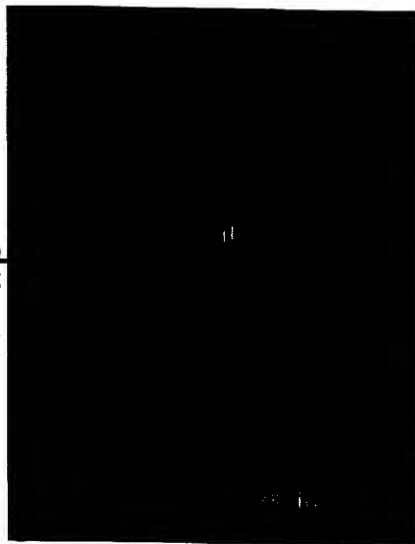
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Anti-Core



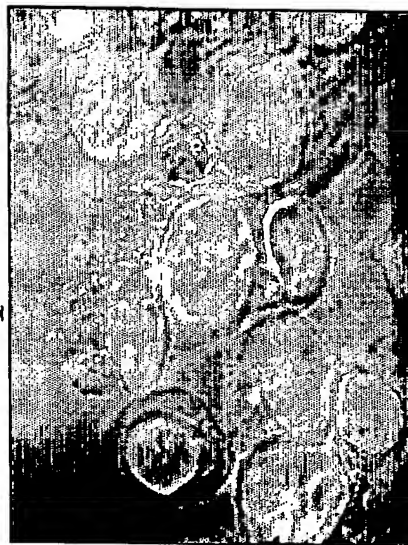
Dapi



Phase



Phase/Dapi/Anti-Core



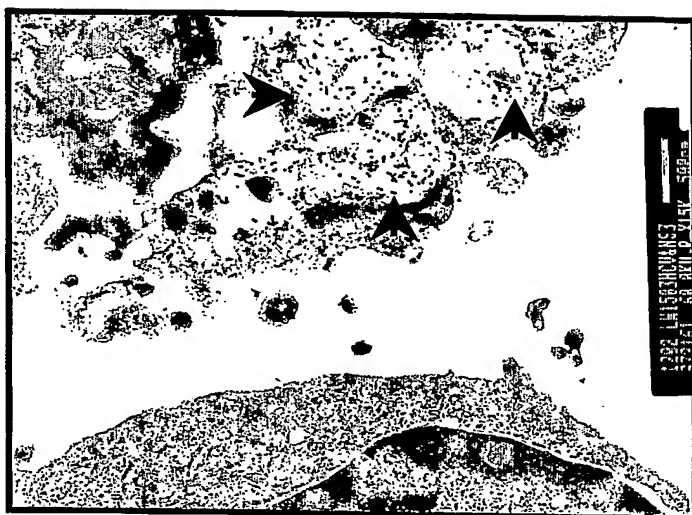
Dapi/Anti-Core



FIG. 35

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五、五

五三

71-36A

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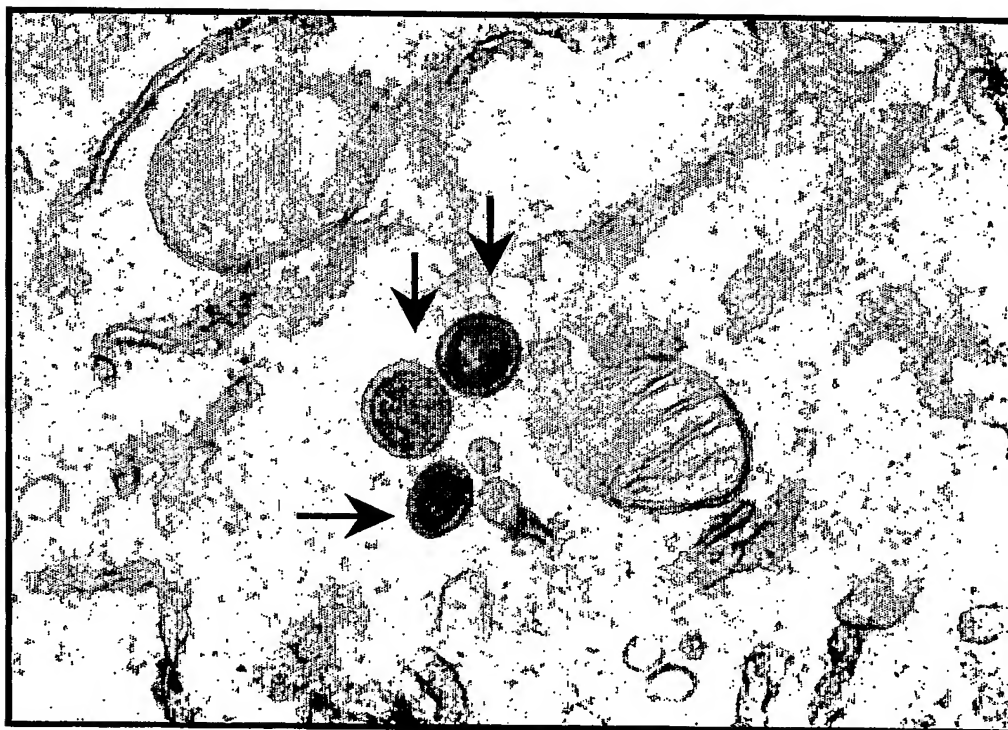
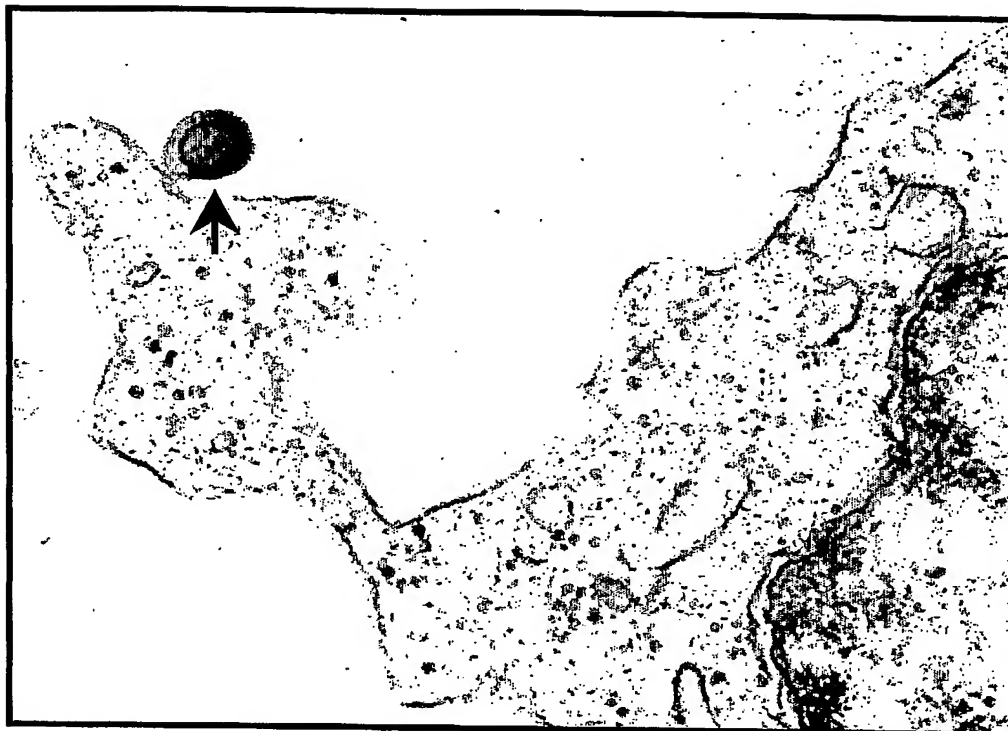


FIG. 37

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Virus partial purification.

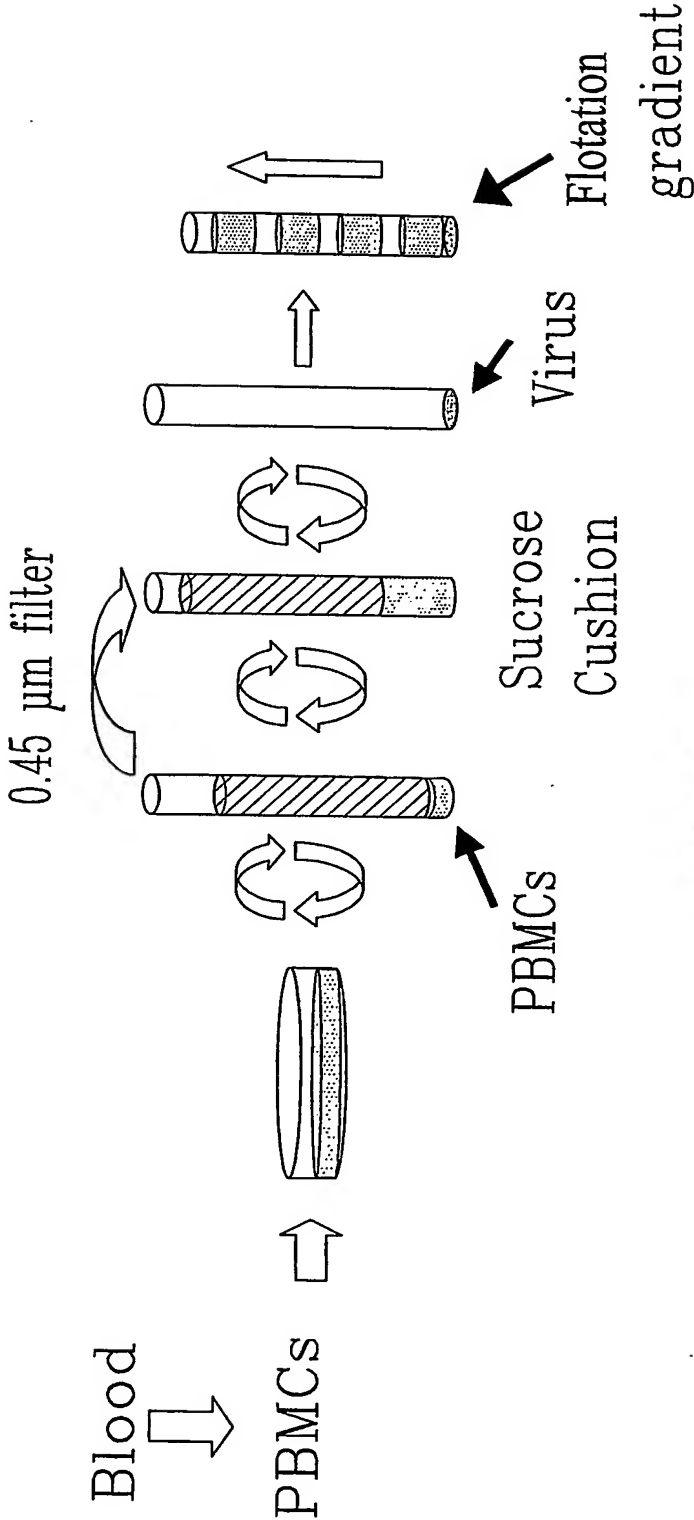


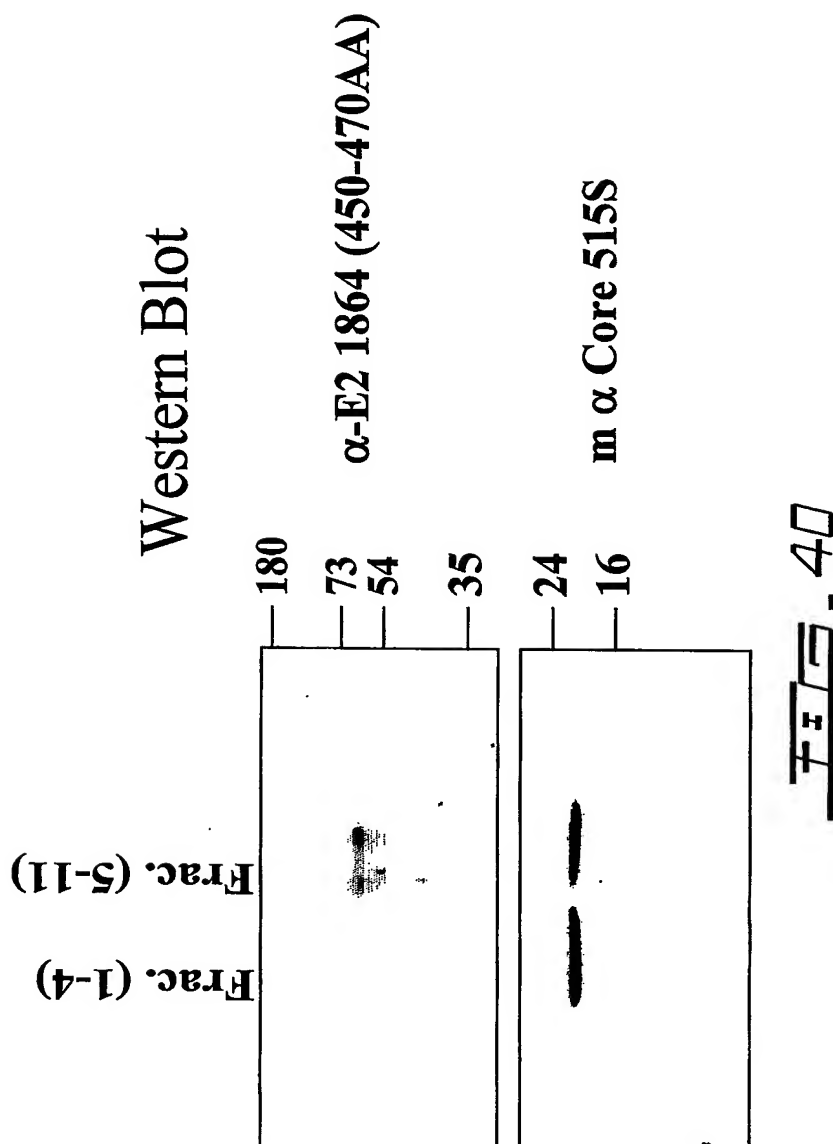
Fig. 3B

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Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen.Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J.Med.Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J.Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J Med Virol (2002) 68, 335
1.11-1.215	HCV(+) PBMCs	-----

Fig. 39

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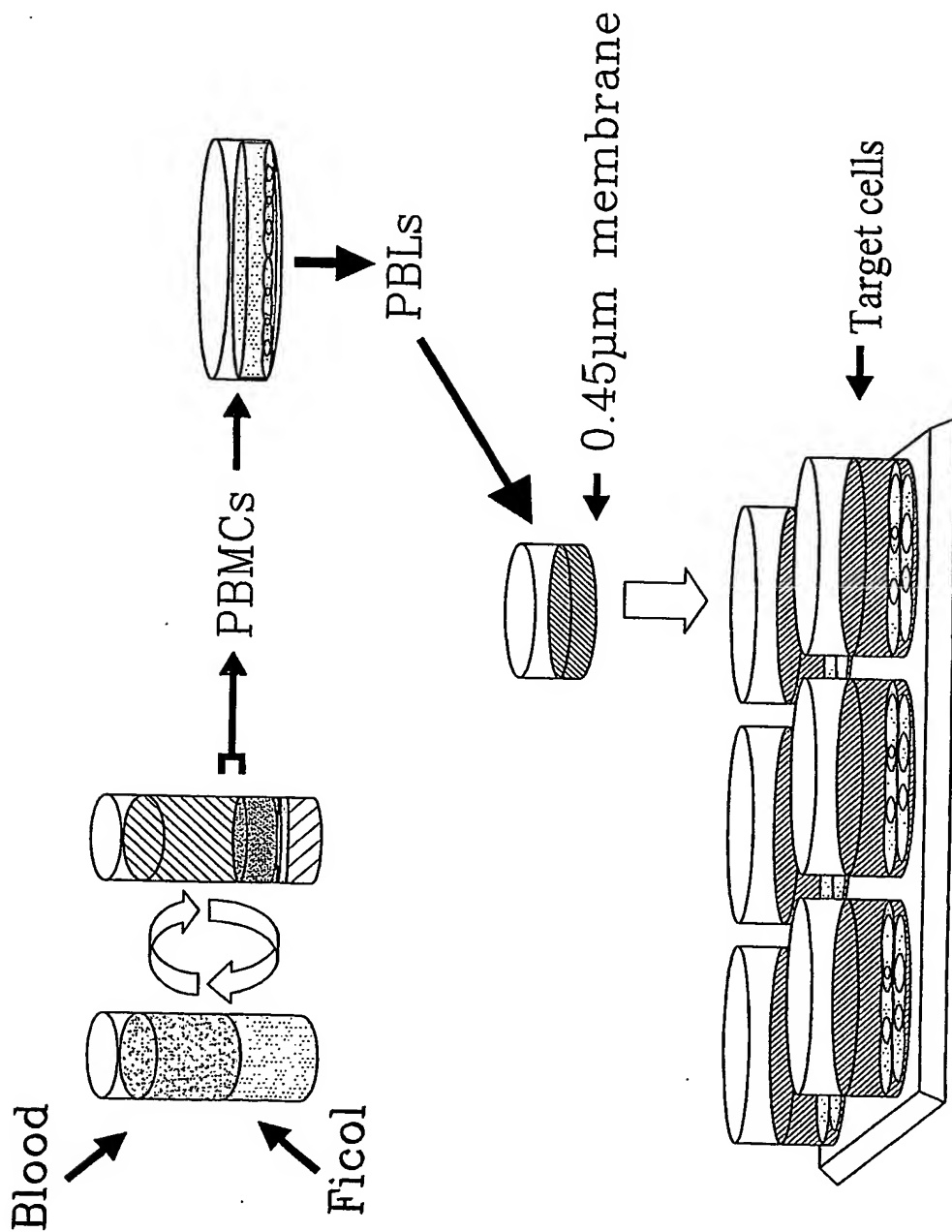
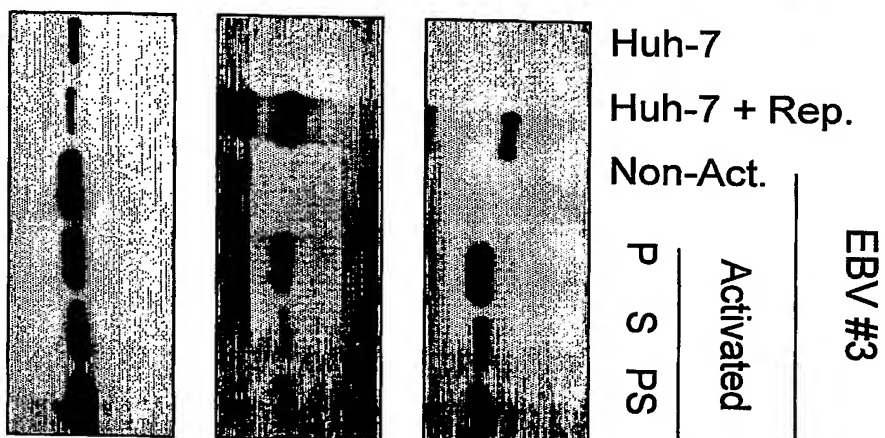
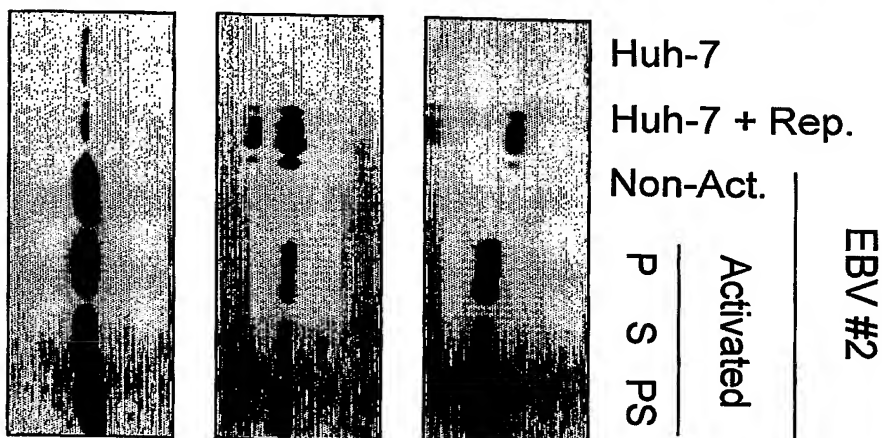
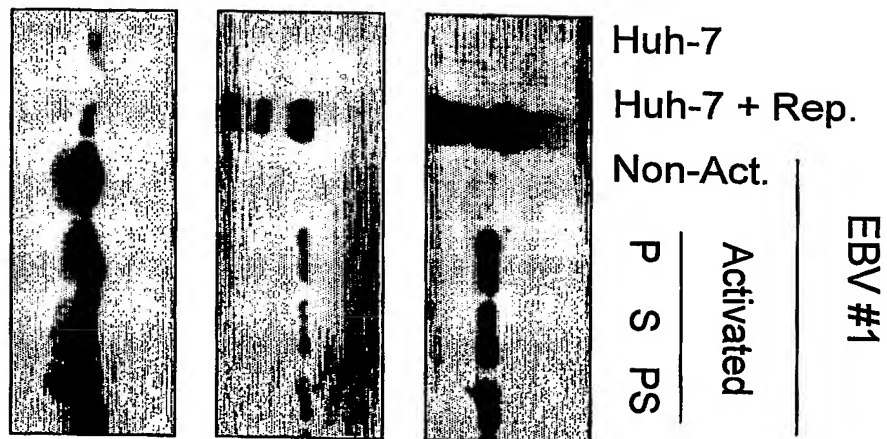


FIG. 41

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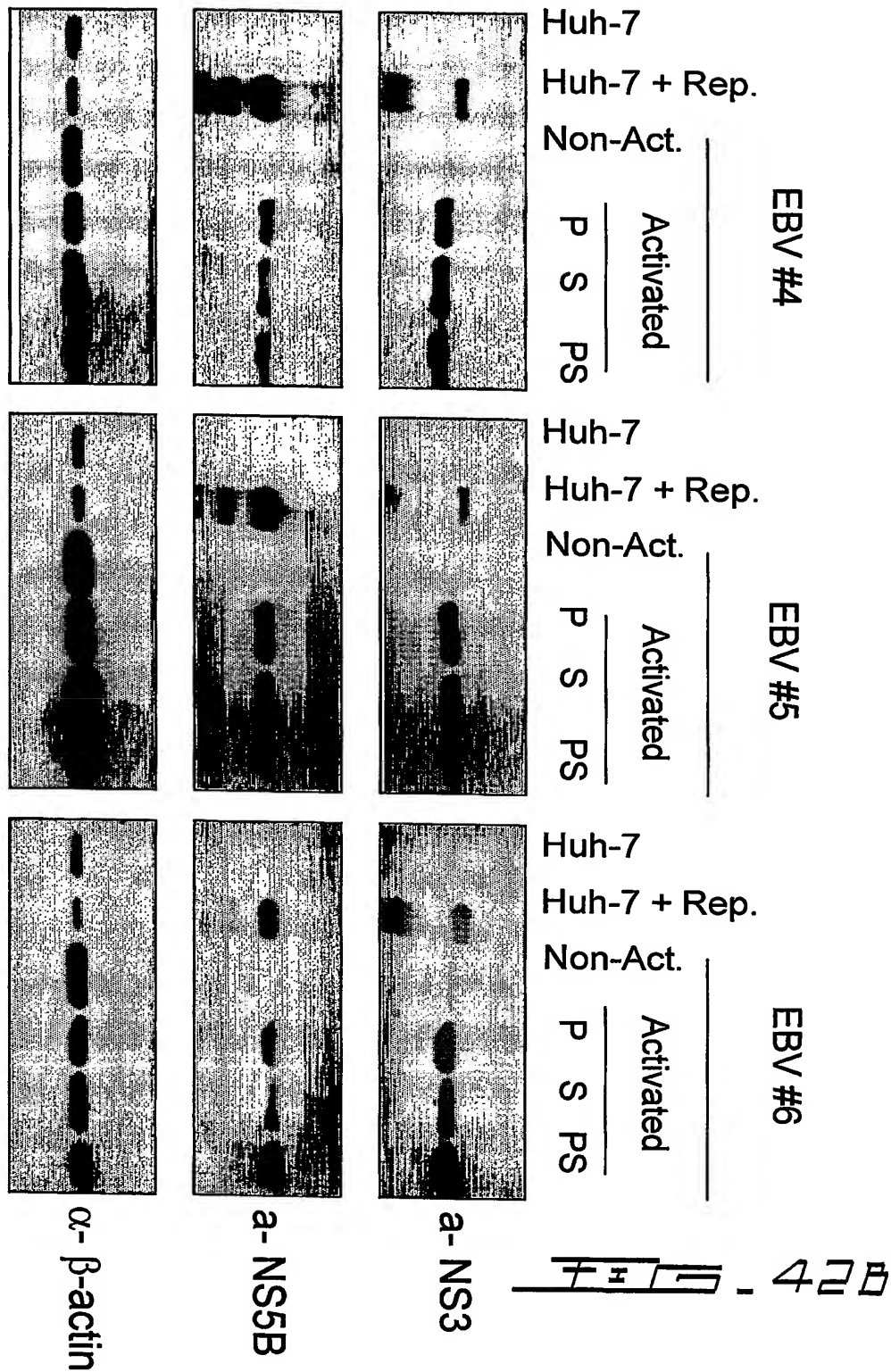
α - β -actin

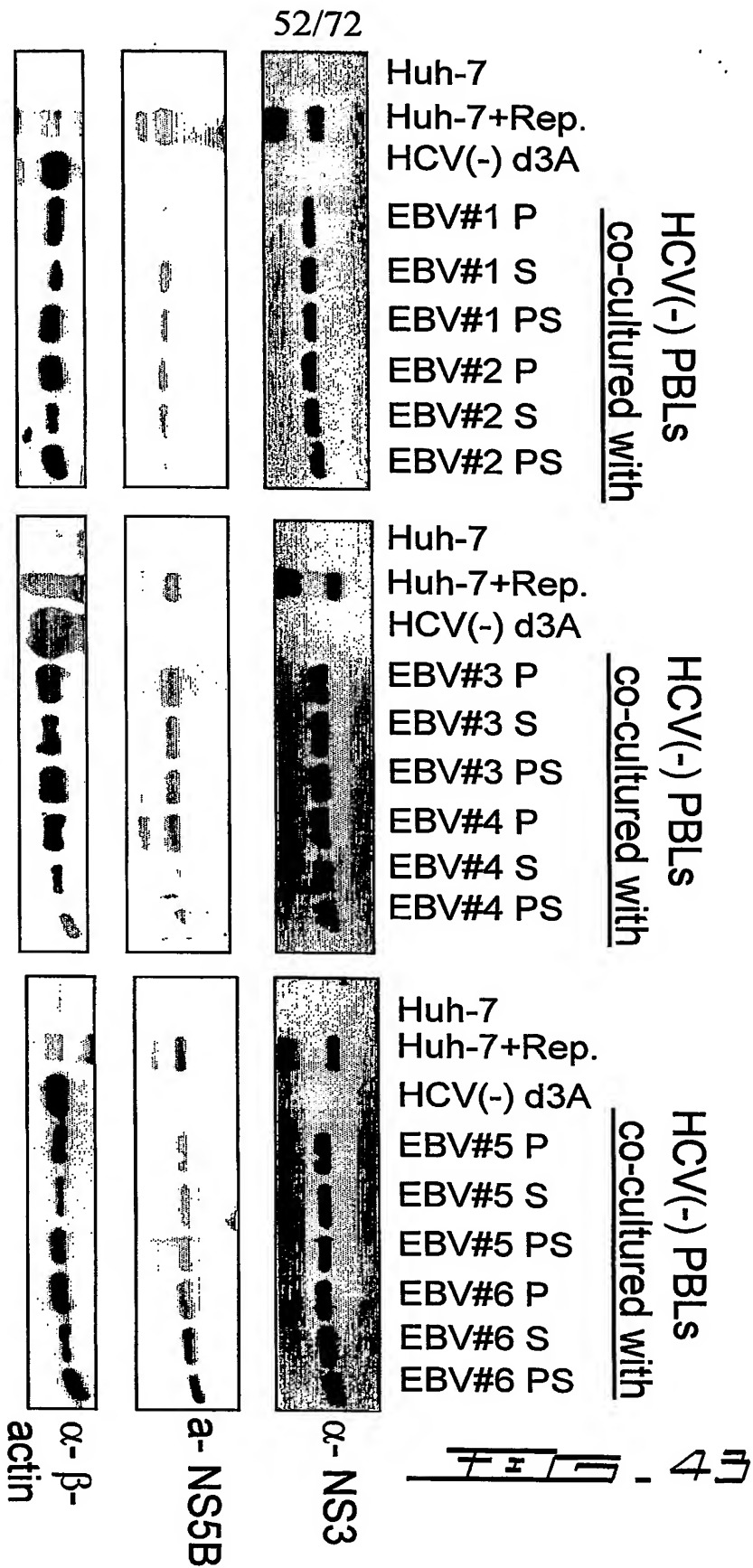
α -NS5B

α -NS3

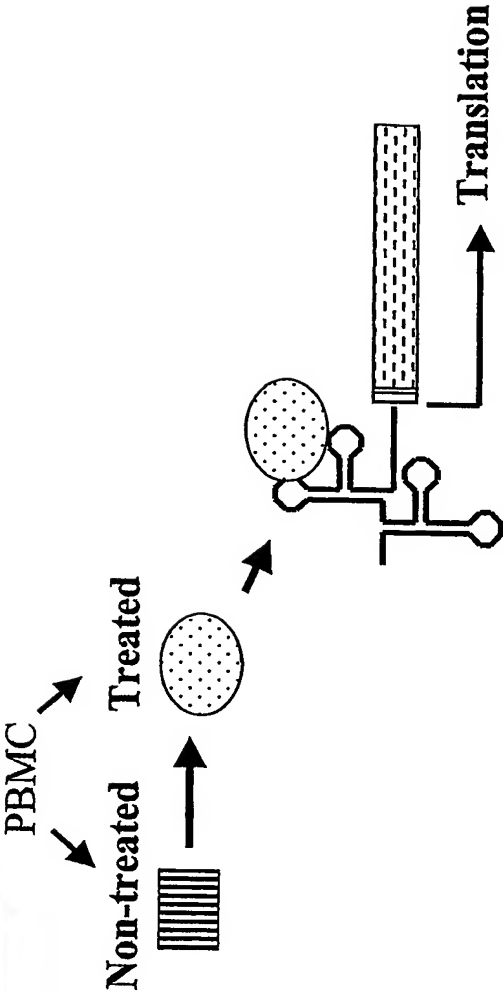
7.42A

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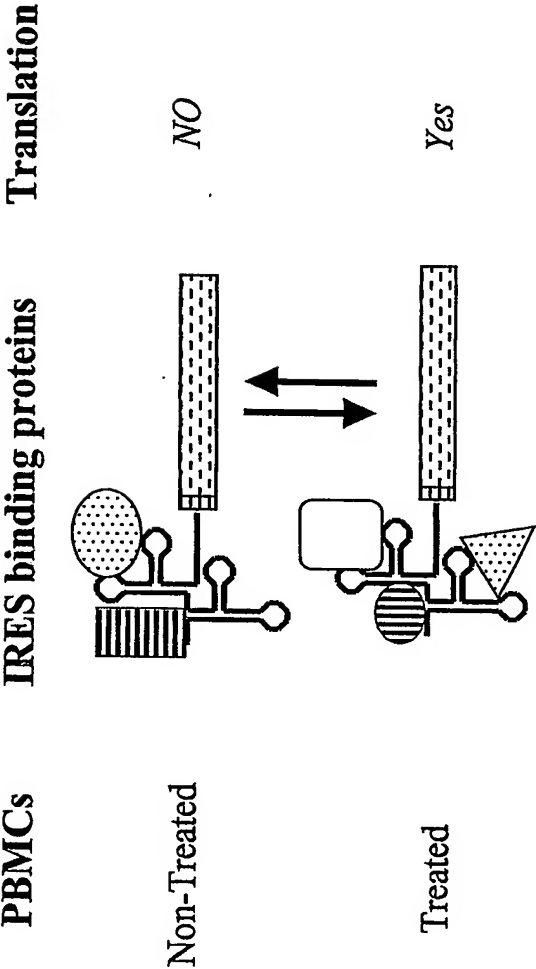


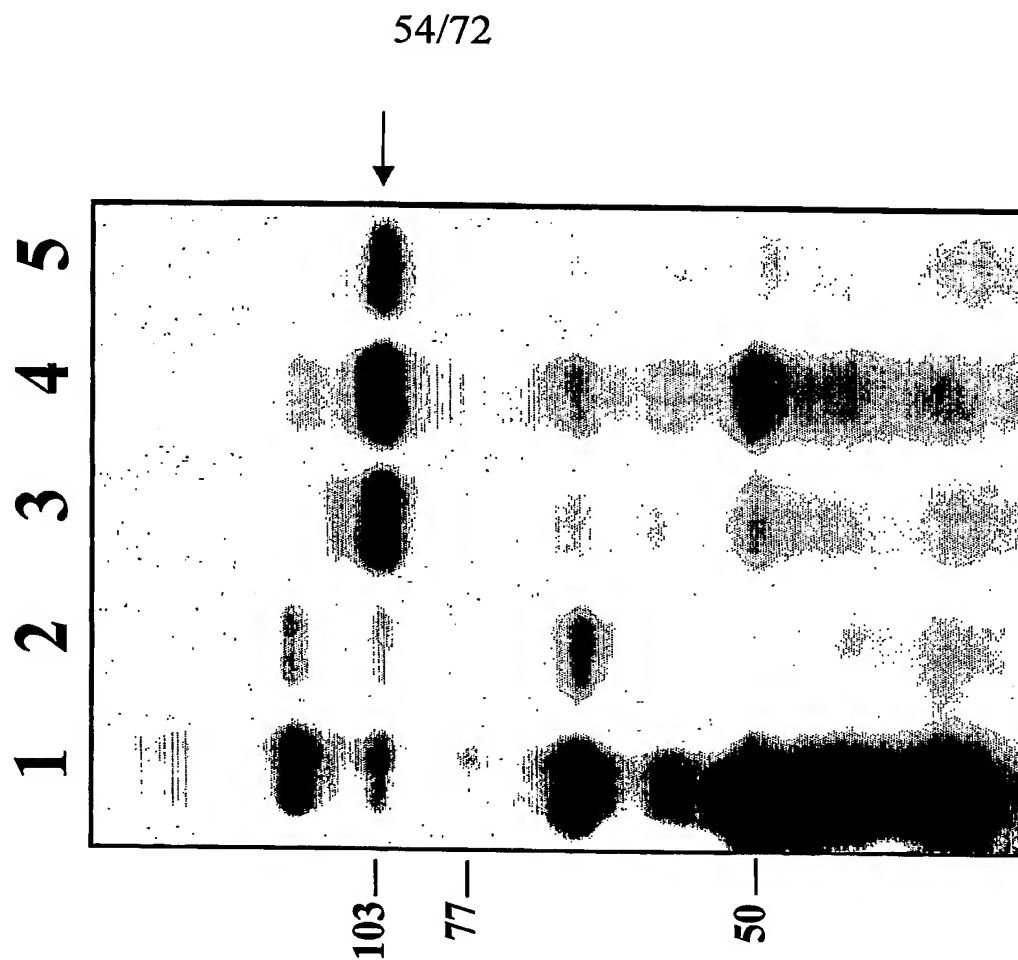


I- Translation Activator.



II- Translation inhibitor.

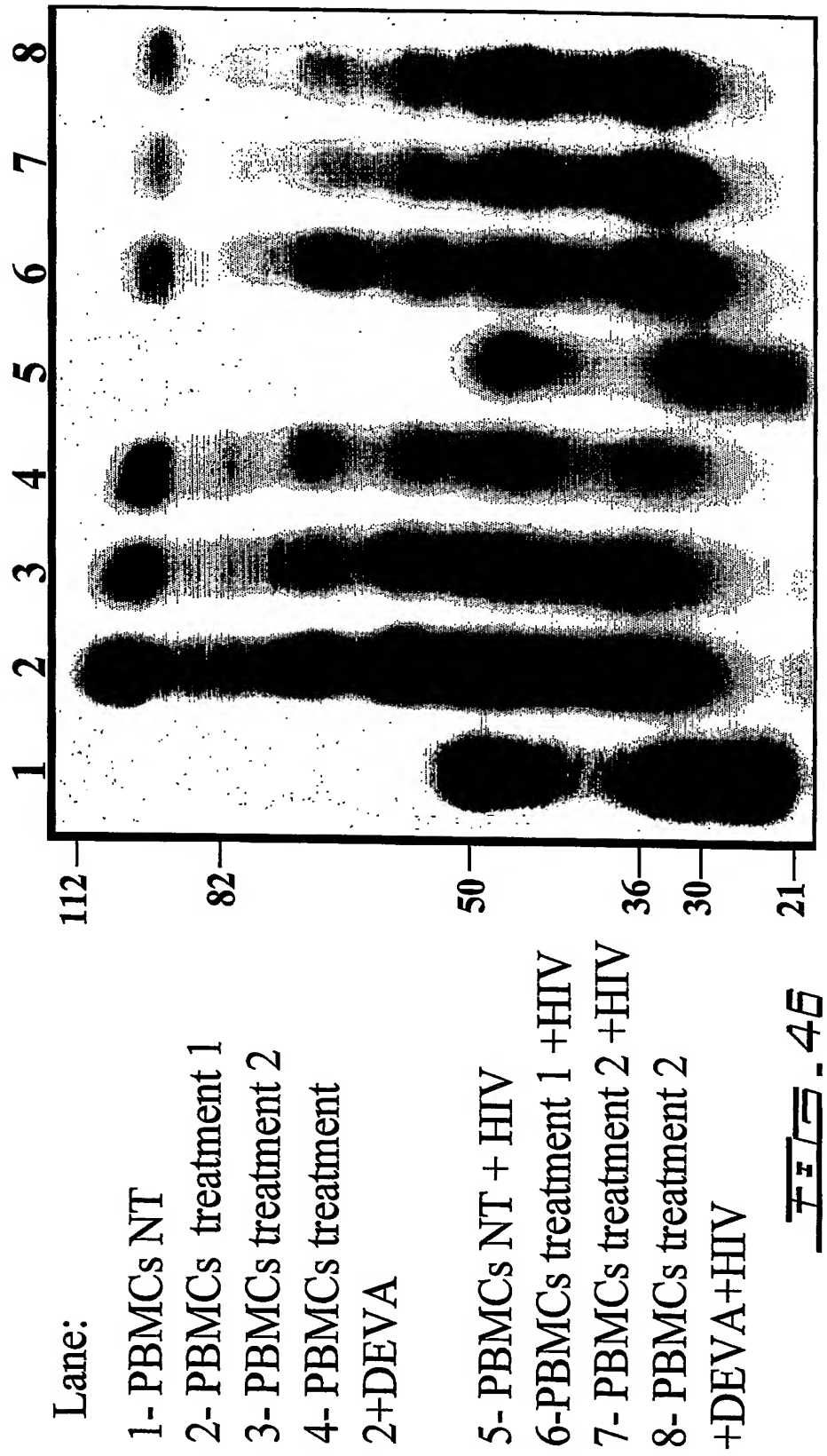


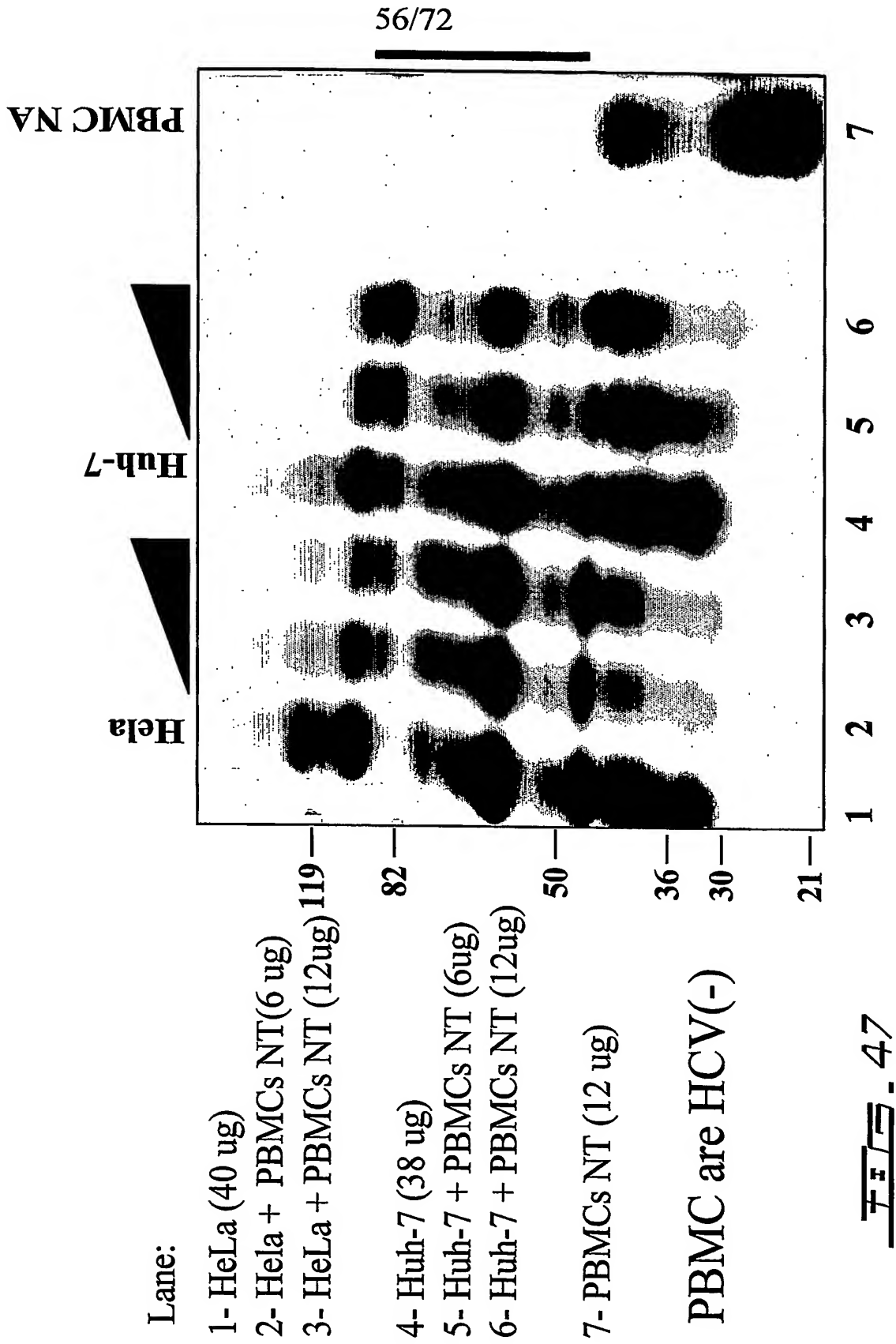


Lane:
 1- HeLa
 2- HeLa + tRNA
 3- HeLa + EMCV IRES
 4- HeLa + polio IRES
 5- HeLa + ODC IRES

FIG. 45

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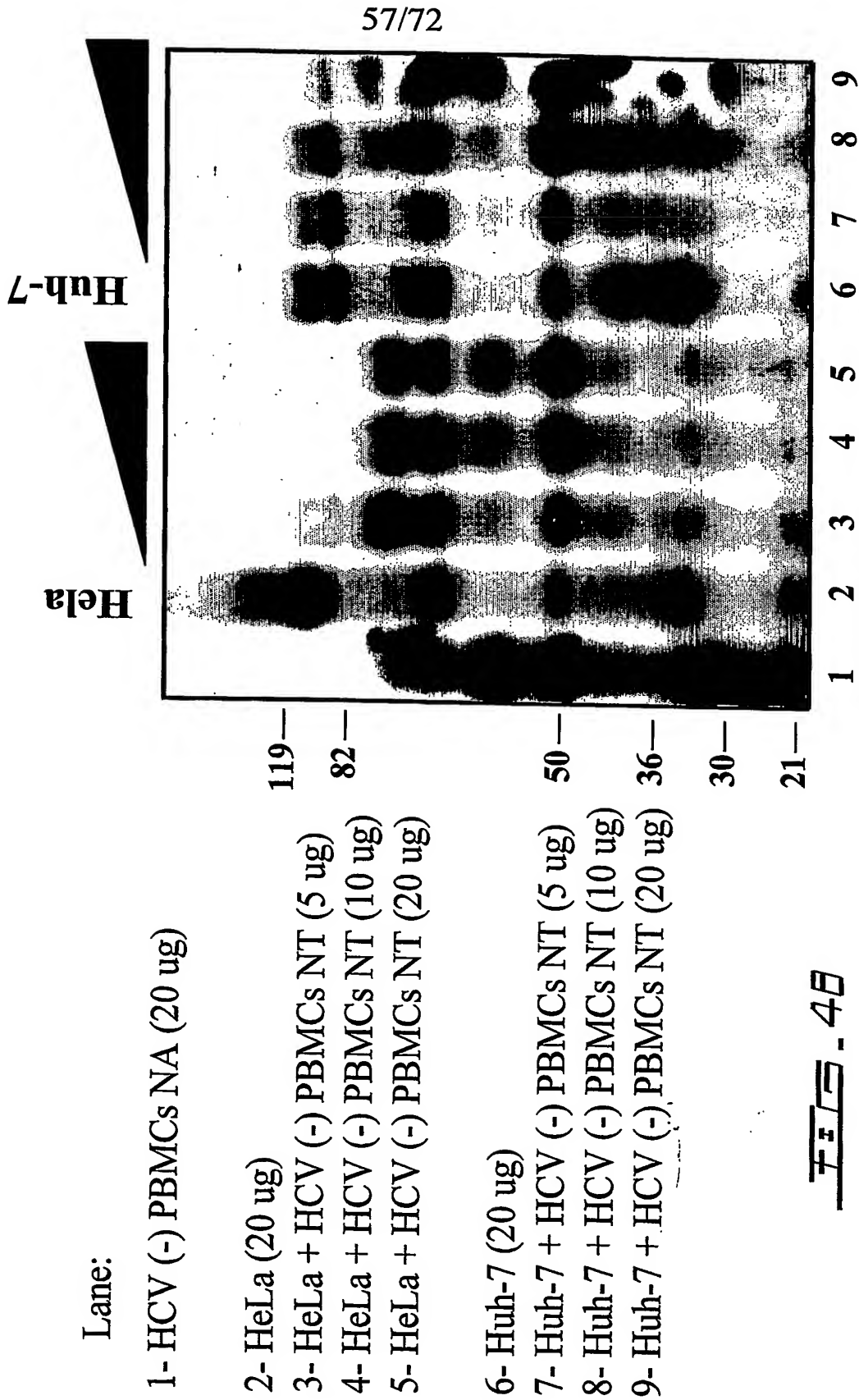
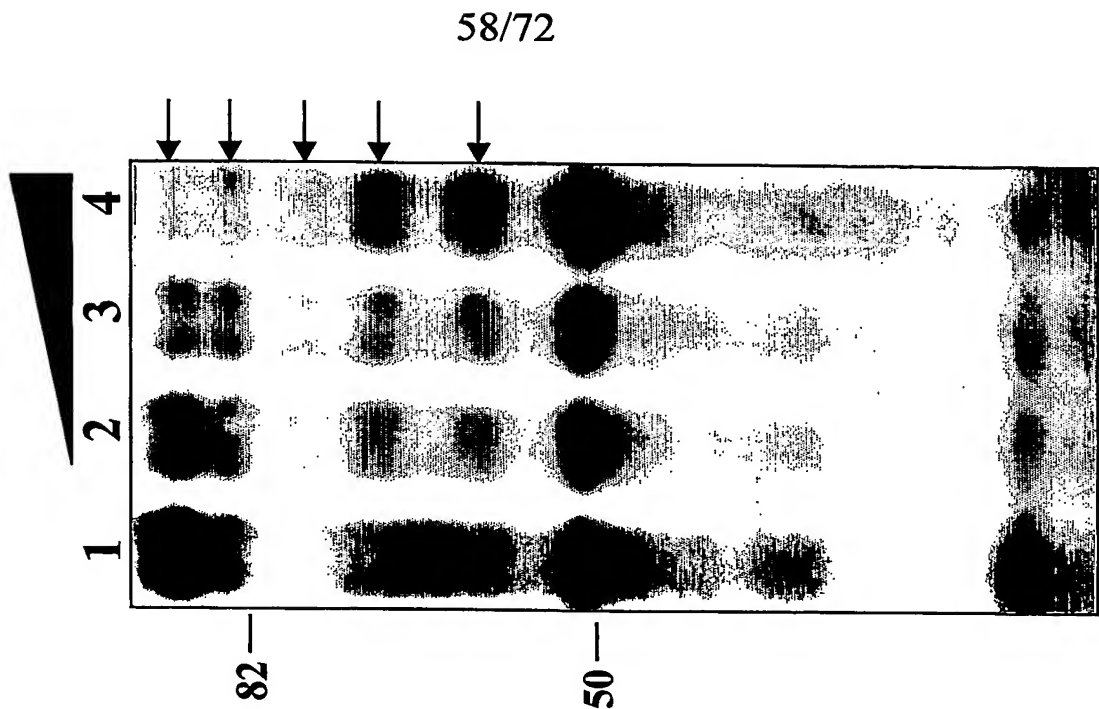


FIG. 4B

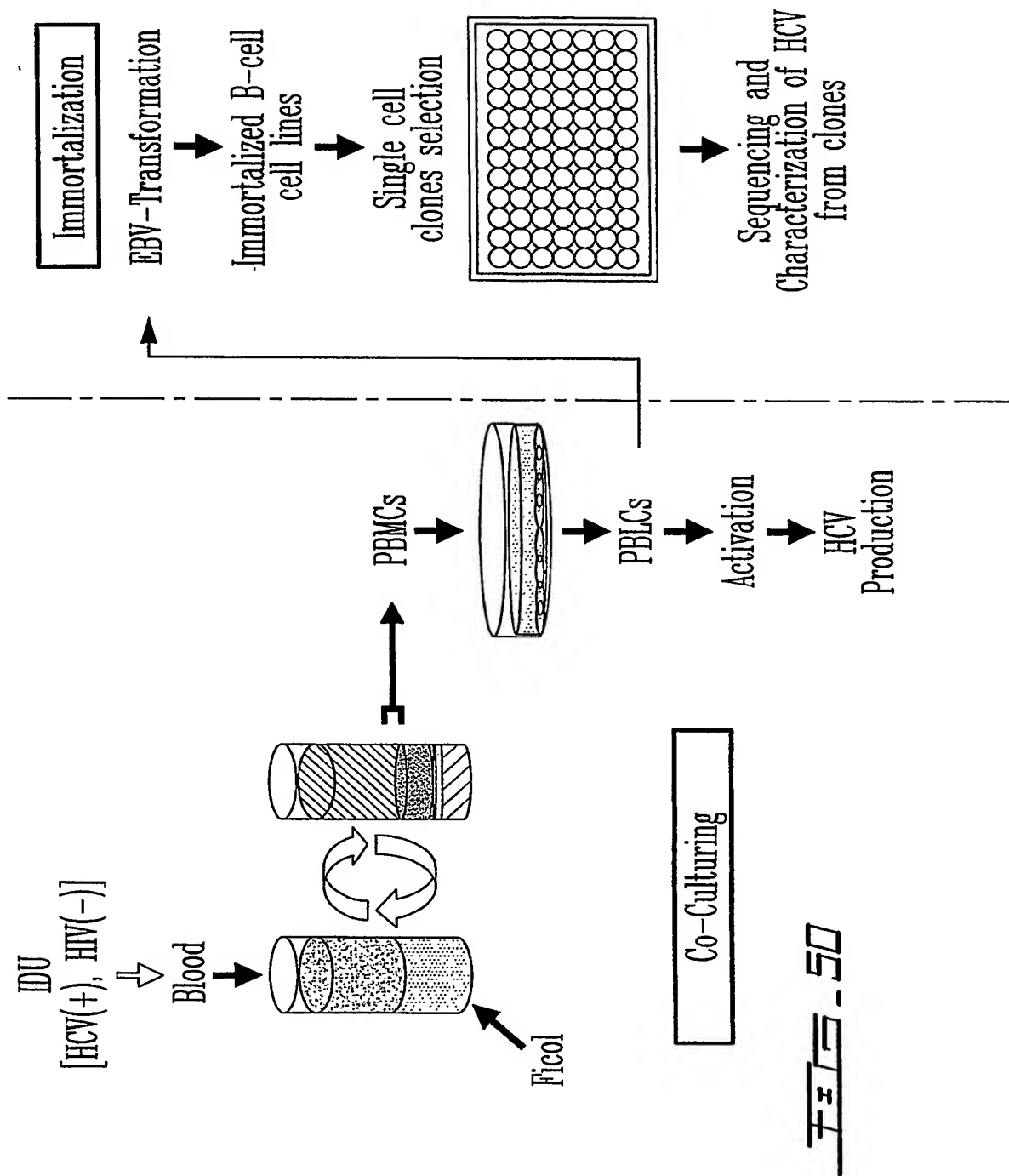


Lane:

- 1- Huh-7 (20ug)
- 2- Huh-7 + HCV (-) PBMCs NT (5ug)
- 3- Huh-7 + HCV (-) PBMCs NT (10ug)
- 4- Huh-7 + HCV (-) PBMCs NT (20ug)

FIG. 49

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HCV(+)- EBV-Transformed B-Cells.

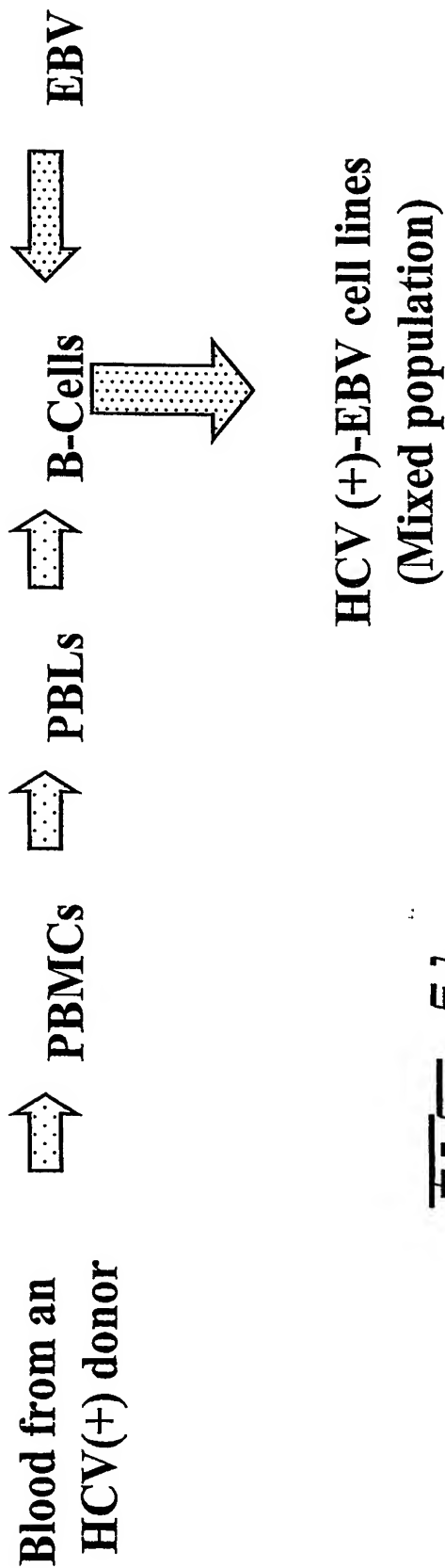


FIG. 51

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HCV RNA is detected in mixed population of EBV-transformed B-cells

HCV (+) Strand RNA

	Non-Stimulated cells	Stimulated cells
Cell line	RNA Copies /10 ⁶ cells	RNA Copies /10 ⁶ cells
EBV-1	4.66x10 ⁵	2.33x10 ⁶
EBV-2	2.77x10 ⁵	7.91x10 ⁴
EBV-3	3.96x10 ⁶	4.02x10 ⁵
EBV-4	2.03x10 ⁶	1.57x10 ⁶
EBV-6	1.41x10 ⁶	4.32x10 ⁵
EBV-HCV (-)	0	0

GAPDH mRNA

	Non-Stimulated cells	Stimulated cells
Cell line	RNA Copies /10 ⁶ cells	RNA Copies /10 ⁶ cells
EBV-1	2.23x10 ⁸	2.19x10 ⁸
EBV-2	8.73x10 ⁸	2.25x10 ⁸
EBV-3	1.83x10 ⁹	1.77x10 ⁹
EBV-4	5.48x10 ⁸	3.79x10 ⁸
EBV-6	1.26x10 ⁹	9.42x10 ⁸
EBV-HCV (-)	9.27x10 ⁷	3.62x10 ⁸

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Control EBV-HCV (-); anti-Core

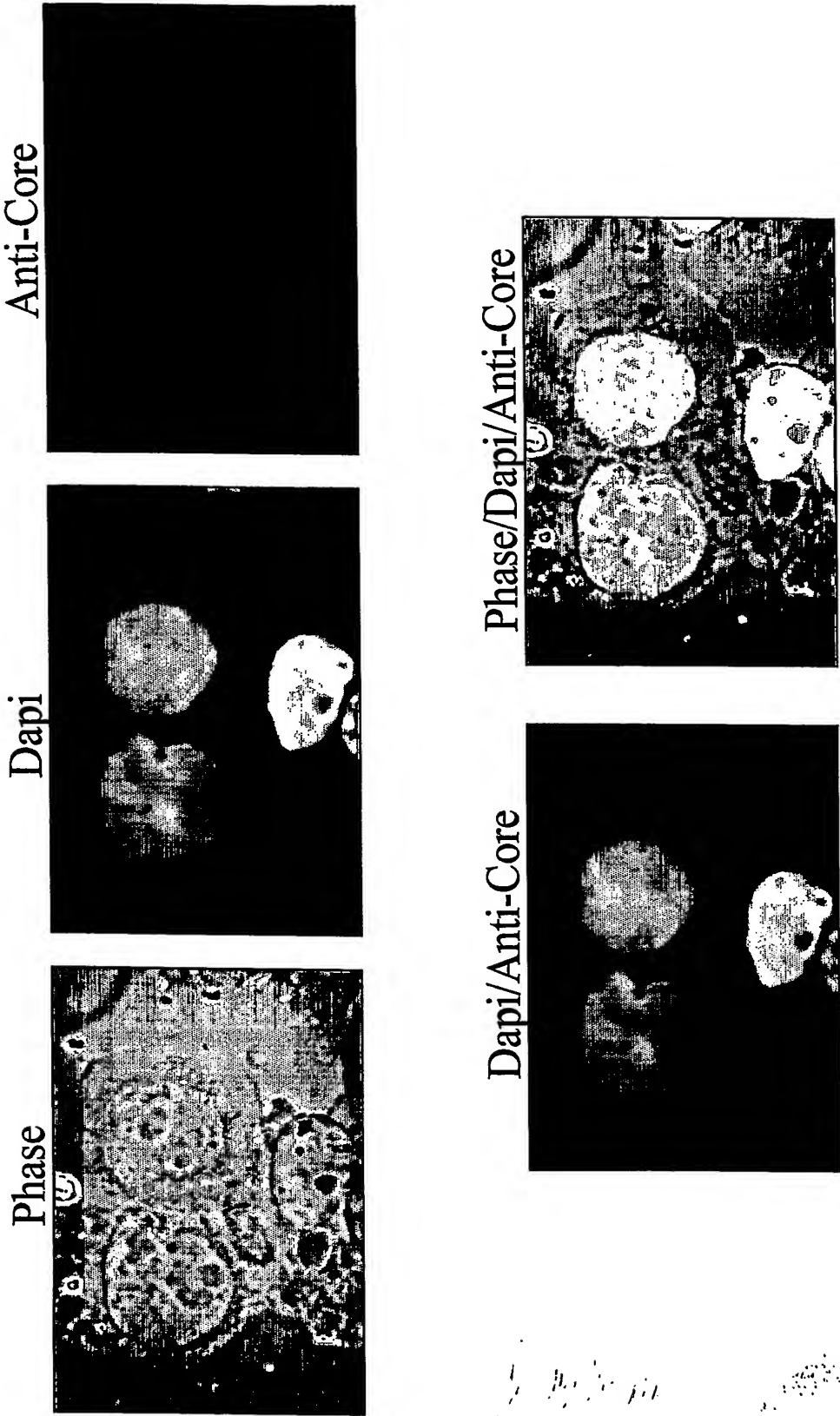


FIG. 53A

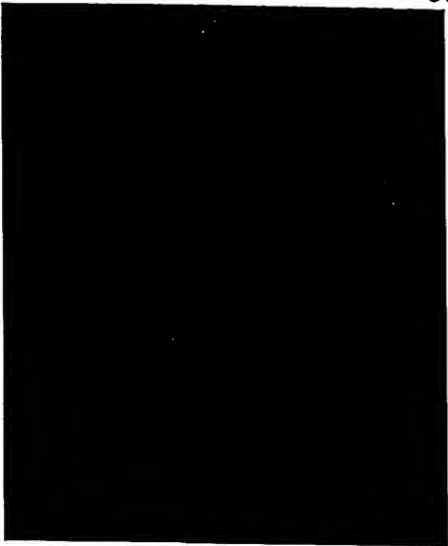
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Detection of Core in EBV-2

Dapi

Anti-Core

Phase



Dapi/Anti-Core

Phase/Dapi/Anti-Core



FIG. 53B

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HCV(+)-EBV-Transformed B-Cells.

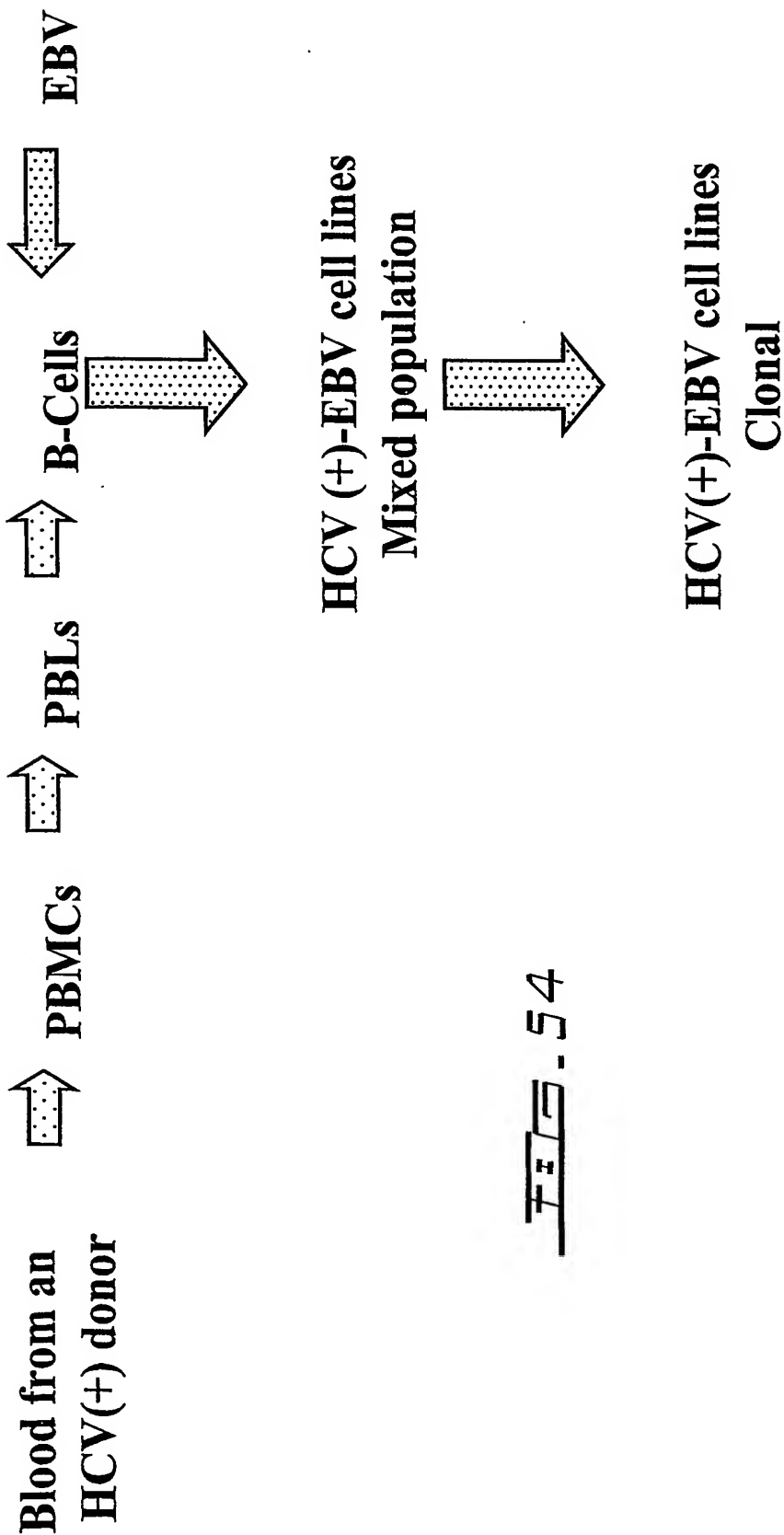
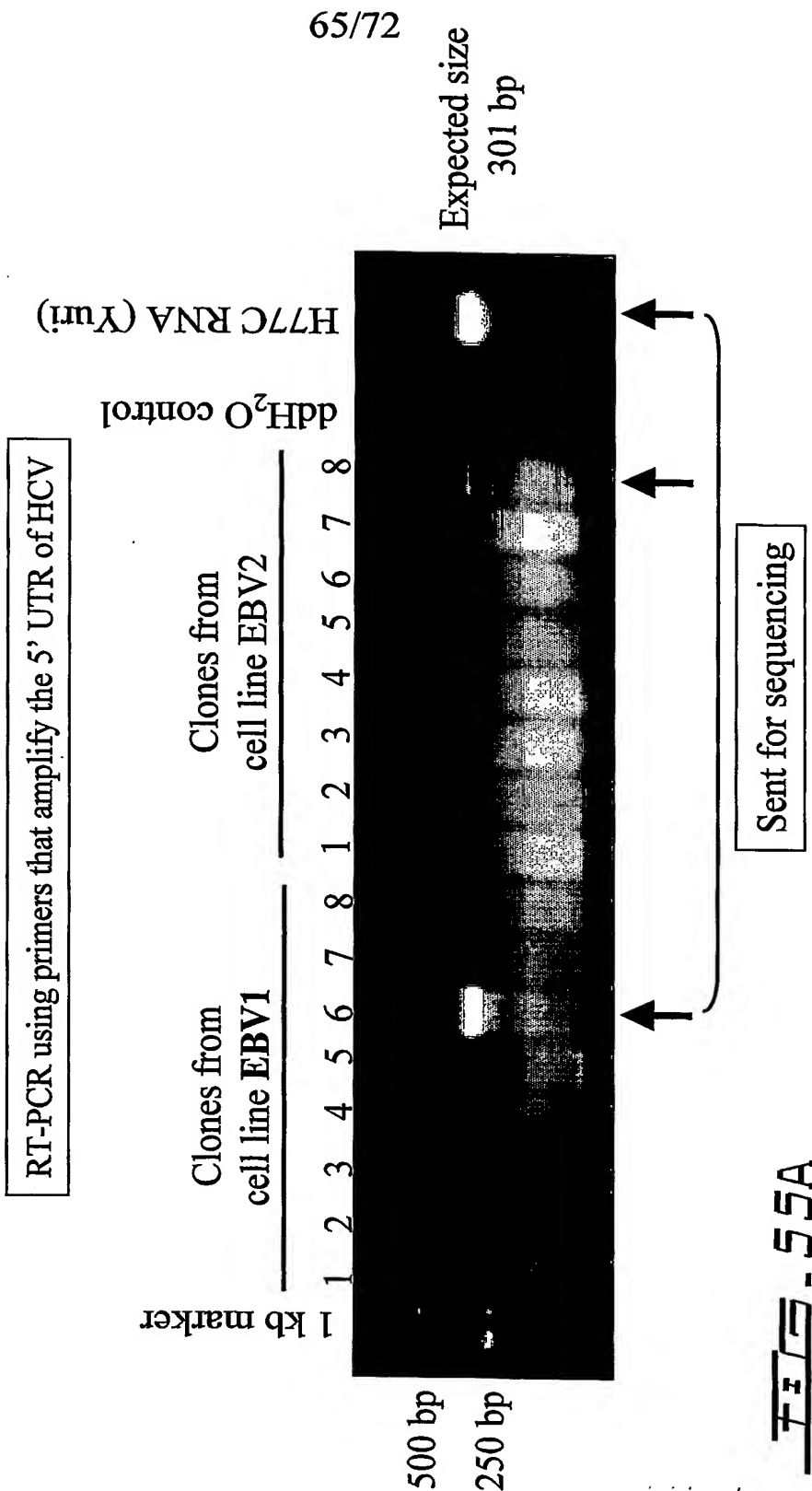


FIG. 54



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Alignment: H77C (RT-PCR positive control) sequence (top)/
EBV1 clone 6 sequence (bottom)

```

CACTCCCCTGTGAGAACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT
CACTCCCCTGTGAGAACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT

TAGTATGAGTGTGTCAGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGGTC
TAGTATGAGTGTGTCAGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGGTC

      G
TGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTCTTGGATAA
TGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATTA

ACCCGCTCACATGCCCTGGAGATTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAG
ACCCGCTCA -ATGCCCTGGAGATTGGGCGTGCCCCCGCGGAGACTGCTAGCCGAGTAG

TGTTGGGTCGCGAAAGGCCCTTGTGGTACTGCCTGATAGGGT
TGTTGGGTCGCGAAAGGCCCTTGTGGTACTGCCTGATAGGGT

```

~~Fig. 55B~~

Blue: sequence from virus in the serum (MLL-005).

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Alignment: H77C (RT-PCR positive control) sequence (top)/
EBV2 clone 8 sequence (bottom).

CCAGGACCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC
CCAGGACCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC

GGTGATACACCGGAATTGCCAGGACCGGTCCTTTCTTGG
GGTGATACACCGGAATTGCCAGGACCGGTCCTTTCTTGG

ATAAACCCGCTCAATGCCCTGGAGATTTGGGCGTGCCCCCGCAAG
ATAAATCCGCTCAATGCCCTGGAGATTTGGGCGTGCCCCCGCAAG

ACTGCTAGCCGAGTAGTGTTGGTCCGAAAGGCCTTGTGGTAC
ACTGCTAGCCGAGTAGTGTTGGTCCGAAAGGCCTTGTGGTAC

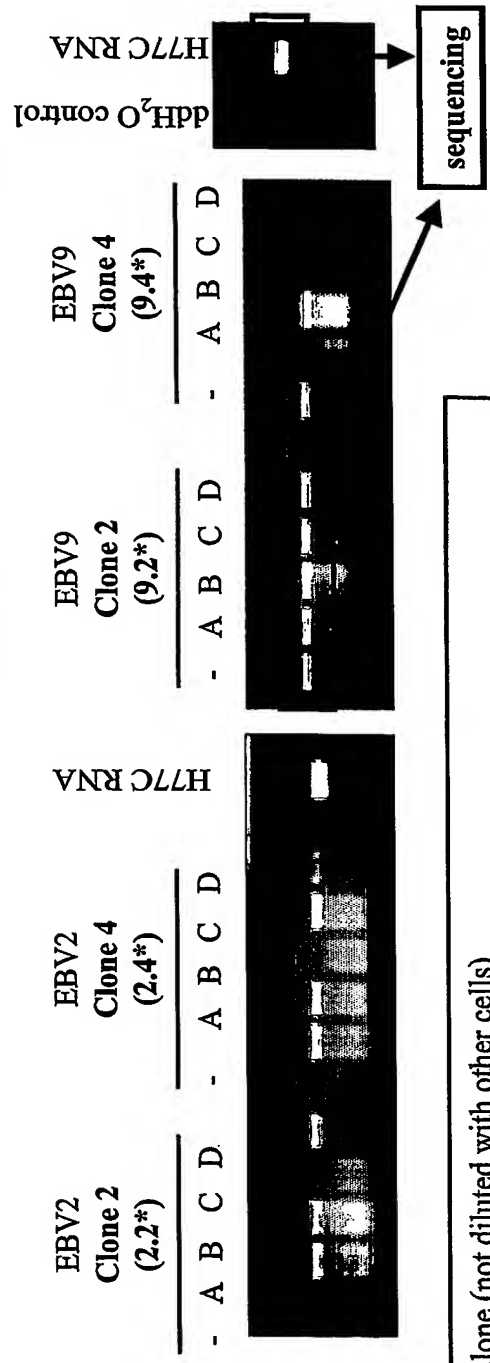
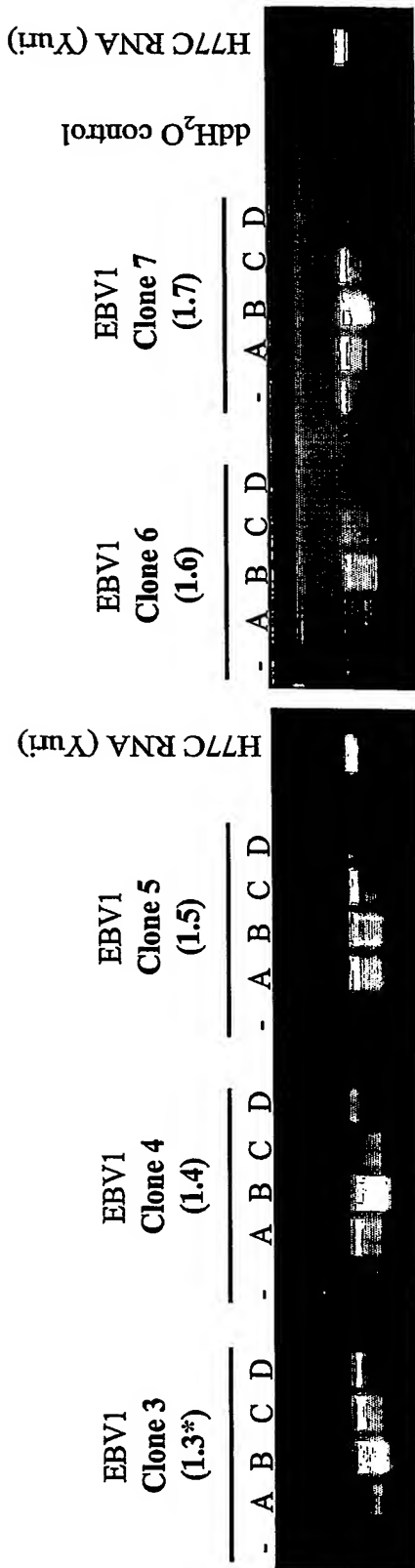
TGCCTGATAGGTGCTTGCAGTGCCCCGGAGGTCTCGTAGAC
TGCCTGATAGGTGCTTGCAGTGCTCCGGGAGGTCTCGTAGAC

CGTGCA

CGTGCA

FIG. 55C

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- = clone alone (not diluted with other cells)
A= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)
B= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)
C= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)
D= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

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Alignment of all 9.2 sequences

H77C CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAAGCGTCT
9.2 final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAAGCGTCT
9.2a final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAAGCGTCT
9.2b final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAAGCGTCT
9.2c final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAAGCGTCT
9.2d final seq CACTCCCCTGTGAGGAACTACTGTCTTACGCAGAAAAGCGTCT

H77C AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC
9.2 final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC
9.2a final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC
9.2b final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC
9.2c final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC
9.2d final seq AGCCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCC

H77C CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTGAGTACAC
9.2 final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTGAGTACAC
9.2a final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTGAGTACAC
9.2b final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTGAGTACAC
9.2c final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTGAGTACAC
9.2d final seq CCTCCCGGGAGAGCCATAGTGGTCTCGGAAACCGGTGAGTACAC

- = clone alone (not diluted with other cells)
a= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)
b= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)
c= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)
d= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

Red= Variation with respect to clone 9.2

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Alignment of all 9.2 sequences

H77C	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATAAACCCGCT
9.2 final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATAAACCCGCT
9.2a final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGAT <u>T</u> AACCCGCT
9.2b final seq	CGGAATTGCC <u>GGGA</u> <u>AGAC</u> <u>T</u> GGGTCCTTCTTTGGATAAACCC <u>A</u> CT
9.2c final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGATAAACCCGCT
9.2d final seq	CGGAATTGCCAGGACGACCGGGTCCTTCTTTGGAT <u>T</u> AATCCGCT
H77C	CAATGCCTGGAGATTTGGCGGTGCCCCCGCAAGACTGCTAGCCG
9.2 final seq	CAATGCCTGGAGATTTGGCGGTGCCCCCGCAAGACTGCTAGCCG
9.2a final seq	CAATGCCTGGAGATTTGGCGGTGCCCCCGC <u>G</u> AGACTGCTAGCCG
9.2b final seq	<u>C</u> <u>T</u> <u>A</u> TGCCCGG <u>C</u> <u>C</u> <u>A</u> TTTGGCGGTGCCCCCGCAAGACTGCTAGCCG
9.2c final seq	CAATGCCTGGAGATTTGGCGGTGCCCCCGCAAGACTGCTAGCCG
9.2d final seq	CAATGCCTGGAGATTTGGCGGTGCCCCCGC <u>G</u> AGACTGCTAGCCG

FIG. 57B

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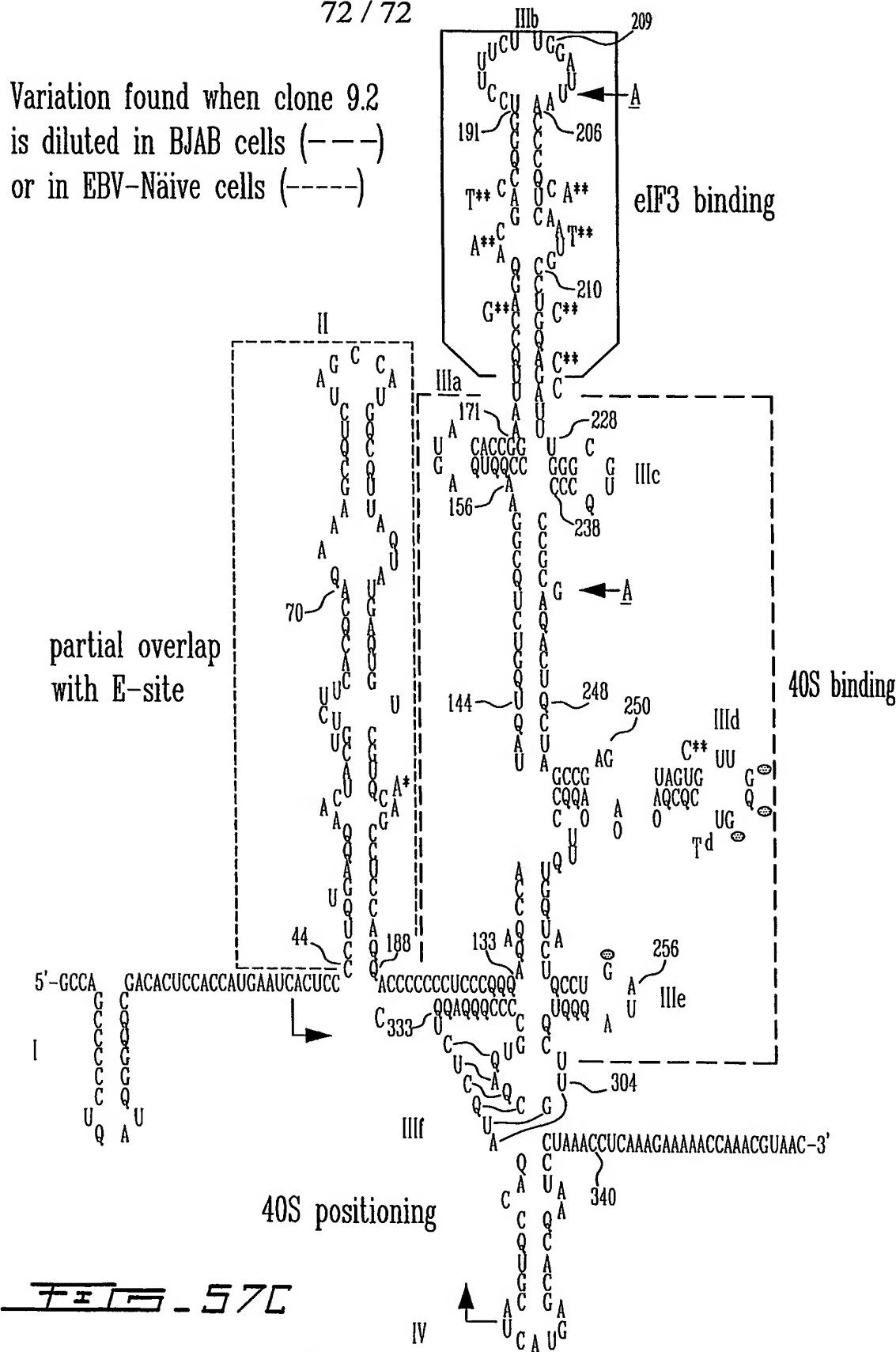
Alignment of all 9.2 sequences

H77C	AGTAGTGTGGGTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2 final seq	AGTAGTGTGGGTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2a final seq	AGTAGTGTGGGTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2b final seq	AGTAG C GTGGGTTGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2c final seq	AGTAGTGTGGGTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2d final seq	AGTAGTGTGGGTCGCCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
H77C	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2 final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2a final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2b final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2c final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA
9.2d final seq	GTGCTTGCGAGTGCCCCCGGAGGTCTCGTAGACCGTGCA

FIG. 57B (Cont.)

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Variation found when clone 9.2
is diluted in BJAB cells (---)
or in EBV-Näive cells (-----)



➔ = Primers

AUG start codon
IRES structure from Sarnow P (2003), J Virol. 77, 2801-6